Morphology, Biology and Control of Fowl Caecal Nematode, Heterakis gallinarum (Schrank, 1788) Madsen, 1949

ABSTRACT

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
ZOOLOGY

By
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DEPARTMENT OF ZOOLOGY
AligarhMuslim University, Aligarh
September 1976

ABSTRACT

Detailed study on the histological anatomy of the adult worm, Heterakis gallinarum (Schrank, 1788) Madsen, 1949, has been made. The important features are, that the body wall comprised of cuticula, sub-cuticula and musculature. Cuticula is found to have only five layers. The dorsal, ventral and lateral chords formed by sub-cuticula which undergo modification in the region of nerve ring, excretory pore, vulva and anus. Musculature is polymyarian and coelomyarian type. Digestive system is a straight tube beginning with the mouth encircled by three lips each with two papillae. Oesophagus is oxyuroid type. It is covered over by tunica propria and the ground tissue comprised of marginal and radial muscles which also enclosed in them the oesophageal glands and nerve tissue. The intestine comprised of epithelial cells and communicates further into the rectum. In female, the rectum forms an exit through the anal pore on the ventral side; but in male a common ano-genital passage, the cloaca is present. Excretory system is simple and comprised of excretory pore, terminal excretory duct, excretory sinus and a pair of lateral excretory canal. The main parts of the nervous system are nerve-ring and four cephalic ganglia. Four main nerves, the so called somatic nerves originating from the respective ganglia proceed

posteriorly in the chords till the hinder part of the body. The dorsal somatic nerve terminates insignificantly, but the ventral nerve forms the pre-anal ganglion and end up into the anal ganglion close to the anus. The lateral somatic nerves form the lumbar ganglia in the caudal region and terminate into the phasmids. In male, five pairs of nerves arise from the lateral somatic nerves and innervate the genital papillae and the sucker. The female reproductive system comprised of vulva, vagina, uterus, oviduct and the ovary. The worm falls in the didelphic and amphidelphous group. Each ovary is divided into germinal zone and growth zone. Uteri are thin walled wide tube and join to form the common vagina which opens through slit like vulva in the equatorial region. Males are monorchic. Testis occupies the anterior part of the body and via seminal vesicle, vas deferens and ductus ejaculatorious open into the cloaca. Accessory organs include, caudal papillae, two unequal spicules and a sucker.

Observations on the life cycle of the worm have also been undertaken. Embryonic development upto the infective stage was traced using different media at variable temperatures. Media containing distilled water and 1% formalin found to be most suitable and development upto the vermiform embryo reached within 13 days. Development in nitric acid was very rapid, whereas in potassium dichromate produced just the reverse results

and in both cases low percentage of embryonated ova was obtained. 30°C was found to be the optimum temperature. High and low range of temperature from this level proved detrimental for embryonation. Hatching of ova in vivo took place in the proximal portion of the intestine within 2-3 hours from the initial ingestion. Second stage juvenile reached the caeca within 168 hours. Later, these entered into the tissue phase, completed the 3rd molt and reappeared in the lumen of the caeca within 222 hours. These passed through the 4th molt within 28-30 hours and typical adults were produced in 19-20 days. The life cycle is normally direct but results obtained present positive role of earthworms and grasshoppers as vector in the transmission of infection. Maximum abundance of the adult worms during rainy season also indicated such a possibility. Pathological aspects of the worm was identified during primary infection. It has been found to produce degenerative changes in the superficial lining of the epithelial cells and glands of the caeca. Breach of muscularis mucosa has also been observed. Studies on natural heterakiasis revealed formation of nodules in the caeca.

Screening of a group of chemicals as part of the chemotherapeutic measures has also been conducted. Phenothiazine

proved to be the most effective drug against this worm, while piperazine adipate and Enheptin-T showed moderate efficacy. These drugs appeared to be comparatively safe as no significant side effects were observed.

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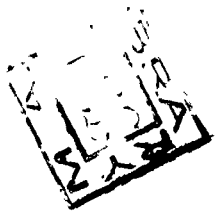

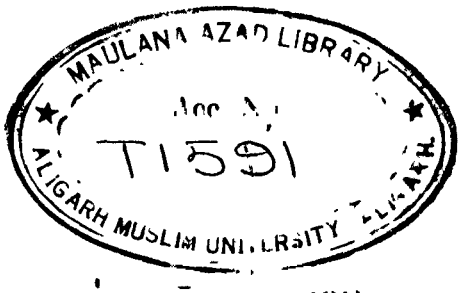

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Date September 20, 1976.

This is to certify that the thesis entitled "Morphology, biology and control of fowl caecal nematode, Heterakis gallinarum (Schrank, 1788) Madsen, 1949." has been completed by Mr. Khurshid Ahmad Alavi under my supervision.

The work is original in nature and independently pursued by the candidate. I have given him permission to submit the thesis for the award of Ph.D. degree in Zoology.

Jamil A. Ansari

(Dr. Jamil A. Ansari)

D E D I C A T E D

TO MY

MOTHER AND FATHER

IN

GRATITUDE

C O N T E N T S

PART - C. Control

ACKNOWLEDGEMENTS

My sincere gratitudes are due to Dr. Jamil A. Ansari for his valuable guidance and selfless help during the progress of this work.

I am specially grateful to Prof. S.M. Alam, Head, Department of Zoology, for providing laboratory facilities and critical suggestions.

I am also grateful to Dr. S. Khera, Joint Director - in - charge, Zoological Survey of India, Dr. R. Tilak, Suptg. Zoologist, Zoological Survey of India and Mr. K.K. Mahajan, Deptt. of Science and Technology, New Delhi for their encouragement. Sincere thanks are also due to Dr. Haroon Ayob, Mr. Mohammad Matin, Mr. Dharam Pal Juneja and Mr. H.S. Mehta for their help in many ways.

The author further wishes to thank Mr. Abdul Baqui and Miss Humaira Khatoon for their help during the course of this work. Thanks are also due to Mr. Mohd. Wazir for his help in the collection of the parasites and rearing of the chickens.

I am particularly indebted to my wife whose constant encouragement provided me incentive, spirit and opportunity to complete this work.

Last but not the least, I am thankful to the Council of Scientific and Industrial Research, New Delhi for financial assistance.

INTRODUCTION

Heterakis gallinarum (Schrank, 1788) Madsen, 1949, is a nematode parasite occurring in the caeca of domestic fowl. The parasite is widely distributed and estimates of its incidence in fowl population in our country usually indicate heavy infection. Besides, many domestic and game birds have also been found to harbour this parasite.

The parasite has been reported as the causal organism of 'Typhlitis' among poultry, which results in diarrhea, consequent loss of condition and death. Formation of nodules and other pathological changes in the caeca have been observed. Evidences are also available that heavy infection with the worm many pre-dispose the host with Leukemia virus. Its role as biological vector in the spread of fatal disease, 'Histomoniasis' among turkeys and fowls has developed considerable interest and opened a new field for scientific investigations.

Poultry farming happened to be a major industry in our country. Rearing of native fowls on non-commercial basis is widely practiced in many villages and towns. Poultry and poultry products sold, used or traded accounts a good part of

of the total income of the country. But the information on various biological characteristics of this worm has been found to be very scanty and incomplete. Obviously, the chances of survival for such a promissing industry appears to be very dim. In view of the alarming situation a thorough study of the worm based on its morphology, biology and control has been undertaken. It is believed that the work will fill in the gap and stimulate interest to our knowledge of the poultry parasites and poultry diseases in India. Since, scientific poultry farming is becoming increasingly important, anything which is learned with regard to the eradication of this parasite is likely to have a world-wide application.

HISTORICAL RESUME

The literature reveals that most of the authors in referring to this parasite used the name Heterakis gallinae (Gmelin, 1790). The author, actually placed this worm with the genus Ascaris and named it, Ascaris gallinae. In 1791, Froelich described it again and called it as Heterakis vesicularis. The parasite was redescribed by Freeborn (1923) who gave the name Heterakis gallinae. Madsen (1949) made a review of the whole literature and brought in some facts regarding the nomenclature of this parasite. The author revealed that Gmelin (1790, p. 3034) based his study of the worm with reference to Goeze's (1782, p. 86) description which later proved to be a species of Ascaridia, probably Ascaridia galli (Schrank, 1788). Goeze, separately described a worm (Plate 1, Fig. 4) which appeared to be the common Heterakis worm of poultry. Schrank (1788, p. 9), with correct reference of description and figures of Goeze, redescribed it under the name Heterakis gallinarum. Hence, on priority basis the proper name for the Heterakis of fowl is now mentioned as Heterakis gallinarum (Schrank, 1788) Madsen, 1949.

The parasite has a wide distribution. Clapham (1933) recorded a host list comprised of representatives from 20 genera and 33 species. The intensity of infestation under natural conditions vary in different parts of the world. A number of workers recorded fairly heavy percentage of infected forms. Dujardin (1845) examined 190 chicken and found 56.8% infested. Ackert (1917) working at Manhattan, Kansas examined 395 chickens and found an incidence of 74.1%. Riley and James (1921) in two separate counts in Minnesota recorded percentage infection by 78.1% and 62.9% respectively. Dorman (1928) examined in two separate groups of 76 and 19 chickens, the first group was found to have a percentage of 68.40. The other group also presented the same percentage of infection. This record is strikingly similar to those of Ackert and of Riley and James. The first group of material came from two possibly different types of environments, the University of Illinois poultry farm and a commercial poultry house, and was collected from 23rd February, 1923 to 10th April, 1926. The second group came from poultry dealers in Rochester, Minnisota and was collected from 4th October, 1926 to 11th November, 1926. It is significant that the data obtained in the two localities were similar and fall between the results obtained by Ackert, Riley and James. Clapham (1933) reported 75.2% infection in chickens and recovered from a single to nearly 700 worms from individual host.

Savchenko (1959), while working in Krinonozoh in U.S.S.R., studied the seasonal dynamics of Heterakis infestation in domestic fowls. He made a monthly examination of ten chickens which showed that in adult birds the lowest infestation occurred in January to March and the highest in July to August, and again it falls in December. In the case of young birds the condition was different, the heterakids were first found in June and highest infestation occurred in August to September, falling again by November., Norton (1964) who worked at the central laboratory, England, reported 77% of the domestic fowls infected with Heterakis gallinarum, with an average number of worms per infested bird was 174, and worm burden ranged from 1 to 3760, the last figure, however, included many juveniles. He also expressed doubt about the occurrence of any seasonal variation.

However, there are only two exceptional reports which give low percentage in the incidence of infestation of this parasite. One is given by Roth (1903) who, while working at Bresban, Germany, examined a total of 230 chickens and reported 13 of it infested with this parasite i.e., an incidence of only 5.6%. Then Hodasi (1969) reported an incidence of only 10.2% and low intensity of infection in the native population of fowls in Legon, Ghana.

Morphology of Heterakis gallinarum has been studied by Chitwood (1931), Clapham (1933) and Baker (1936); and except the last named author, rest have given brief descriptions of the worm. The study made by Baker is the only available literature on the anatomy of this worm.

Considerable difference of opinion has been found regarding the development and life cycle of Heterakis gallinarum. First and most profound observation on the development of ova, under natural conditions, and using both solid and liquid media, was made by Graybill (1921). He observed, that the critical range of temperature for the development of egg in culture of salt solution and sugar was 8 to 11°C. Eggs at room temperature i.e. 19 to 21°C developed into complete embryos within 8 days, while other cultures at 18-26°C produced complete embryos between 8-12 days His list on the resistance of undeveloped eggs to cold, indicated that development of egg continues once they are removed from freezing conditions to room temperature. Embryos kept in salt solution at room temperature survived 12 months, while fully developed ova kept in soil under natural condition retained living embryos even after a period of 8 months. Uribe (1922) made comparable observations on the temperature requirements for the experimental incubation of the eggs of Heterakis papillosa and expressed complete agreement

with those of Graybill. He also added valuable information that the ova developed uninjured in 1.5% nitric acid and the media kept the ova bacteriologically sterile thereby preventing entry of Histomonas pathogen. Dorman (1928) made a detailed study on the cleavage and segmentation of ova of this worm. According to his observations the cleavage is equal, 32-cell stage is completed within 4 or 5 days at 19-21°C. Blastula is formed in 16-cell stage, invagination is initiated in 32-cell stage with the formation of ectoderm, endoderm and blastopore. The most complete study on the development of ova of Heterakis gallinarum was made by Clapham (1933), who reported that development is completed in 14-17 days under favourable conditions. A fairly wide range of temperature from 20 to 30°C is tolerant. The development is also normal in various dilute solutions of acid and disinfectant and suggested the use of 1% formalin which kept down the bacterial growth. Eggs remain viable in such solution even for 7 months. Roberts (1937) reported that the development to the infective stage is reached in 5 days if incubated at 33°C. Wickware (1940) examined the effects of freezing temperature on the embryonation and found ova retaining the infectivity even upto 172 days in such conditions. Osipov (1957) reported the ova reaching the infective stage in 78 days when kept at 10-15°C under laboratory conditions; and the period was

reduced to 6 days at 35°C. Lund et al. (1958) studied the effects of media, viz., physiological saline, 1.5% nitric acid, 1% formalin and 2% potassium dichromate on the embryonation of Heterakis gallinarum ova and its subsequent development in the chickens. All permitted satisfactory embryonation, but the number of worms recovered from chickens fed to eggs from the dichromate solution was less than 1% of the number of eggs administered from the 3 other media. The percentage of eggs which embryonated was highest in 1% formalin.

Considerable variation exists regarding the course of development of Heterakis gallinarum within the host and completion of its life cycle. Generally it is believed that the life cycle is direct, development of the juveniles taking place in the lumen of the caeca. Few have suggested complication in the development processes and there are authors who believe the definite involvement of some vectors in the completion of its life cycle. Leuckart (1876) was the first to initiate the investigation on the life cycle of this worm. He fed eggs which had incubated about 120 days to a chicken and on autopsy after 31 days, about 15 specimens were recovered. Galli-Valerio (1898) and Leutulle and Marotel (1909) reported about the possible formation of cyst in the caecal wall. Riley and James (1921) and Graybill (1921) found that the embryos hatch out in the small intestine and development is

completed by the 24th day. Uribe (1922) expressed his view that the worms obtained 56-61 days after the ingestion of the ova were considered to represent the complete adult stage. Presence of a stage of parasitism within the tissue of the host during the course of larval development has been suggested by Monnig (1927), Dorman (1928) made his observations that the host gets infection by ingesting ova which have undergone incubation and adult worms capable of producing ova may be removed in about 36 days. Itagaki (1930) concluded from the results of his experiments that the juveniles hatch in the proventriculus in 36 hours and begin to penetrate the caeca on the 4th day. Some of the juveniles penetrate the caecal glands, form nodule and attain maturity. He explained that development to the adult stage is preceded by an early migration of the juveniles through the subserous and muscular coats. Baker (1933) studied the post embryonic development and expressed general agreement with earlier workers. The author, further, indicated that the variations in the normal development was due to the appearance of Histomonas pathogen in the host. The most complete study on the life cycle of this worm was made by Clapham (1933) who explained that the first stage which was a rhabditiform larva remain confined within the shell. First molt occurred in the shell and the infective stage embryo with a filariform oesophagus developed.

Hatching took place in the caeca and 2nd molt occurred after 48 hours of the initial infection. After about 96 hours, 3rd molt took place and differentiation continued. Fourth molt was completed on 10th day and young adults emerged out typical in all morphological respects, and eggs could be found in the faeces at about the 24th day from the initial infection.

The author also investigated the possible migration of juvenile in the host tissue during the course of their development as reported by earlier workers, and discarded it completely. Migration of juveniles within the host was reviewed by Madsen (1962) who concluded that the juveniles instead of having a normal course of development enter into a tissue phase involving the mucosa and submucosa layers and later return to the gut lumen.

Indications are also available about the completion of the life cycle involving an intermediate host. Frank (1953) had shown that Musca domestica and Lucilia sp. can carry embryonated eggs of the worm from one host to another mechanically. He has also demonstrated experimentally that the grasshoppers which are eaten in large number by turkeys can carry the eggs in their gut for 96 hours and can initiate development. Recently Lund et al. (1966) reported that the earthworm can transmit infection among turkeys serving as vector.

Much of the literature is available on its association with diseases. It was observed by Riley and James (1921), Van Es and Martin (1923), Beach and Freeborn (1930) as the causal organism of Typhlitis among poultry. Morgan and Wilson (1938) reported 'Tuberculosis' of poultry due to this parasite. Clapham (1938) noted advance stages of Leukaemia in fowls with heavy infection of this worm. Dauglieva (1966) noted the haematological changes in the blood of the host in a way that the amount of the total protein decreased and there was an increase in globulin fraction simultaneously.

Graybill and Smith (1920) reported that enterohepatitis or black-head, a highly infectious and fatal disease, caused by protozoan parasite, Histomonas meleagridis, could be induced by feeding young turkeys with embryonated eggs of Heterakis gallinarum. Their suggestion was conferred by Tyzzer (1934) who reported the identification of the protozoan in the epithelial cells of the gut of a young nematode. Under field conditions population of Heterakis nearly always carry Histomonas pathogen, as the protozoans are capable of surviving only for short duration outside in the poultry farms, unless they are localized in worm's egg. Majority of the hosts thus become infected with the parasite, so much so, that according to the

estimates of Lund (1958) about 1000 ova are sufficient to induce Histomoniasis in atleast 90% of the fowl population.

There are conflicting reports on the association of the juveniles and the worms with the wall of the caeca and its glands. Most of the workers believe that the larvae remain in intimate association with the wall of caeca and some of these actually penetrate into the mucosa layers which leads to the formation of nodules; whereas, few are of the view that the worms do not have any tissue phase at all.

First report on the formation of caecal nodules due to this parasite was given by Itagaki (1930) while making an aetiological study of these nodules. The author observed that in early stages of their formation the juveniles penetrate the sub-mucosa from the caecal glands and encyst in the muscular layer where these continue to grow. Baker (1931) also reported the formation of nodule in the caeca of fowl infected with Heterakis and suggested that the initial cause of nodule formation may be parasitic or bacterial, the irritant acting on the caecal glands of the mucosa led to the thickening, which later transformed into larger nodule formation. But Clapham (1933) expressed doubt about the formation of any lesions or nodule in the caeca due to this worm. Further, Wickware (1947) while working on the same problem observed that even in the case of caeca free from

'Heterakiasis' the caecal lesions were present which led him to believe that the nodules were not caused by the worm but due to some other reasons not known.

Grigorev (1959) studied the pathogenecity of Heterakis infection in detail and described the formation of two types of nodules in the caeca of the fowl, one is the uncomplicated nodules lymphoid or lymph glandular type, and other nodule complicated by Heterakis infection. The affected mucosa showed infilteration of lymphoid cells and necrosis of the epithelial cells. In the liver, hypernemia of the vessels, hyperlasia of the lymphoid cells and eosinophilia were observed. He believed that some of the adults enter in the already formed nodules and thus cause atrophy and necrosis of the epithelial cells. Meads and Taylor (1963) examined caeca of the birds infected with Heterakis and found it enlarged and snd spotted with lesions, each containing an immature worm. They also suggested vitamin A defficiency as a contributing factor in the production of these lesions. Nath and Pande (1963) have reported erosion of caecal wall due to the presence of adult worm. Their information is based on the study of 'Heterakiasis' in nature. Kaushik and Sharma Deorani (1969) demonstrated the formation of nodule during their study on the tissue response of Heterakis gallinarum infection in chickens.

Because of the absence of precise knowledge concerning the life processes of Heterakis gallinarum, very little information is available on its control and other preventive measures. A number of chemicals were utilized in the control operation and with the exception of phenothiazine which was partially successful, no other drug initiated a dependable efficiency. First report on the use of phenothiazine was given by McCulloch and Nicholson (1940) who suggested that 0.05 to 0.5 grams is a satisfactory individual dose with average effectiveness ranging from 95 to 100% and that the large and repeated doses are non-toxic. Olivier et al. (1943) were of the view that an intake from 0.5 to 1.0 grams of phenothiazine within 6½ to 7½ hours to individual poults is necessary for complete removal of the worm. Guthrie and Harwood (1942) observed that tablets containing 33 parts of phenothiazine, 66 parts of nicotine bentoniteand 1 part of sodium stearate were found to be very effective in removing most of the worm from the host.Wehr and Olivier (1946) reported the inability of phenothiazine to prevent maturation of the worm, and is effective in expelling the worm only when these attain maturity. A new chemical Enheptin-T was introduced by Horton-Smith (1951) against heterakids infection. Anthelmintic activity of Piperazine citerate was tested by Shumard and Eveleth (1955) against Heterakis gallinarum

infection and was found to have little efficacy. Larson and Hansen (1957) also found piperazine to be least effective against heterakids, but gave promising results in reducing the incidence of typhlitis. Sub-cutaneous injection of 90% w/v solution of methyridine in water at the rate of 1 ml per 10 pound body weight was advised by Fernando and Jayasinghe (1963) as an effective control measure against this infection. Schanzel and Hubacek (1964) reported a 0.5% aqueous solution of Methyridine as highly effective when given orally. Fedotova (1969) used Hygromycin B as an anthelmintic against Heterakis gallinarum and found 75 - 90% effective.

SYSTEMATIC POSITION

Heterakis gallinarum (Schrank, 1788) Madsen, 1949, has been included in the superfamily Oxyuroidea Railliet, 1916; with Heterakidae as one of the four families reported from birds viz., Oxyuridae Cobbold, 1864; Heterakidae Railliet et Henry, 1914; Aspidoderidae Freitas, 1956 and Subuluridae Yorke et Maplestone, 1926.

Family Heterakidae Railliet et Henery, 1914

Family diagnosis: Oxyuroidea

Mouth with 3 or 6 lips or without lips. Terminal excretory duct usually short, buccal capsule absent. Males with one or two spicules. Females normally with two ovaries. Development usually direct and without intermediate host.

Sub-family: Heterakinae Railliet et Henry, 1912.

Diagnosis: Heterakidae

Mouth is provided with distinct lips, cuticle smooth or transversely striated. Oesophagus divisible into two parts, a short narrow anterior portion which leads into a well developed bulb. Caudal alae present. Spicules unequal. Sucker is

present with sculeritized rim. Musculature polymyrian.

General characters of the genus Heterakis Duj., 1845:

The genus Heterakis includes worms of small size. The body of the worm tapers on both the extremities. The anterior end is flexed in most of the cases in both the sexes. Body cuticle show inconspicous transverse striations throughout its length. Mouth opening is circular or tri-radiate with three lips. Each of the lips are provided with two papillae. The mouth leads into a well defined oesophagus. The oesophagus is club shaped having a short narrow anterior portion which leads into a swollen posterior portion ending into a bulb. The latter opens into a straight intestine through the oesophago - intestinal valve. The excretory system is of the H type with more or less reduction of the anterior branches. The nerve ring is present in the anterior part of the oesophagus. Sexes are separate. Adults live in caeca and also in the intestine of birds, reptiles and mammals.

M A L E:

In male the caudal alae is modified into a chitinous pre-anal sucker. The caudal papillae appear to be somewhat variable in number. There are 10 - 15 pairs of costiform

caudal papillae but their arrangement is not constant in different species. Spicules are paired, equal, sub-equal or unequal without accessory piece. Gubernaculum absent. The right spicule is long with a blunt point while left is short, sharply curved and pointed.

F E M A L E:

The posterior extremity of the female is pointed. The caudal alae is absent. The vulva is present almost in the middle of the body or infront of it. Uterine branches pass in opposite directions. Adults are oviparous, eggs with a thick shell and a clear granulation at the poles.

The genus Heterakis includes a large number of species distributed in various parts of the world. Various species are listed below:

√ Heterakis alata Schneider, 1866
Heterakis altaica Spaul, 1929
Heterakis arquata Schneider, 1866
√ Heterakis bancrofti Johnston, 1912
√ Heterakis beramporia Lane, 1914
Heterakis bonasae Cram, 1927
√ Heterakis bosia Lane, 1914
√ Heterakis brevispiculum Gendre, 1911

Heterakis caudata Linstow, 1906

Heterakis caudebrevis Popowa, 1949

Heterakis chenonettae Johnston, 1912

Heterakis circumvallata Linstow, 1906

✓ Heterakis dispar (Schrank, 1790)

Heterakis fariai Travassos, 1913

Heterakis hamulus Linstow, 1906

Heterakis hastata Chandler, 1926

Heterakis hyperborea Swinyard, 1931

✓ Heterakis indica Maplestone, 1931

Heterakis interlabiata Ortlepp, 1923

✓ Heterakis isolonche Linstow, 1906

✓ Heterakis kurilensis Oschmarin, 1949

Heterakis lanei Chandler, 1926

Heterakis lingnanensis Li, 1933.

Heterakis longespiculum Maplestone, 1931

Heterakis macroura Linstow, 1883

Heterakis meleagris Hsu, 1957

Heterakis monticelliana Stossich, 1892

✓ Heterakis nattereri Travassos, 1924

Heterakis neoplastica Wassink, 1917

Heterakis oscari Travassos, Pinto et Muniz, 1928

Heterakis parisi Blanc, 1913

✓ Heterakis parva Maplestone, 1931

Heterakis pavonis Maplestone, 1931

Heterakis pedioecetes Mawson, 1956

Heterakis psophiae Travassos, 1913

Heterakis pusilla Linstow, 1906

Heterakis putaustralis Lane, 1914

Heterakis silindae Sandground, 1933

Heterakis skrjabini Cram, 1927

Heterakis spiculata (Cobbold, 1861)

Heterakis tenuicauda Linstow, 1883

Heterakis tragopanis Lal, 1942

Heterakis travassosi Khalil, 1932

Heterakis valdemucronata (Molin, 1860)

Heterakis valvata Schneider, 1866

Heterakis variabilis Chandler, 1926

Heterakis vulvolabiata, Chandler, 1926

Heterakis yama dori Yamaguti, 1941

Heterakis vesicularis (Froelich, 1791)

Teixeira de Freitas (1956) regards this species as identical with Heterakis gallinarum (Schrank).

Heterakis gallinarum (Schrank, 1788) syn. Heterakis gallinae Gmelin, 1790; Heterakis longecaudata Linstow, 1879.

MATERIALS AND TECHNIQUES

Source of parasite and host:

Collection of the worm was made from fowls covering an area around the city of Aligarh and its suburbs. The collection was made regularly and five days in a week. A monthly record of the male, female adult worms and juveniles, thus isolated, was also maintained to study the seasonal variation. Each bird was sacrificed and its alimentary canal and caeca were thoroughly examined for the parasite. The worms were picked out, identified and counted. In many cases number of worms recorded was so large that a dilution counting method had to be employed. Later, alimentary canal and the caeca were cut into parts and each segment was separately examined. The mucosal surface was rubbed carefully between fingers to remove any worms adhering to it. The worms were later hot killed and brought to 70% alcohol for preservation. Gross body structures were studied from whole mount preparations of the worm in glycerine.

The chickens used for experimental purposes were of white Leghorn breed and were reared in the parasite free

conditions in the laboratory.

Microtechnique:

For the study of histological anatomy, worms were fixed in Bouin's picric acid and serial sections were obtained using paraffin microtechnique. Different cytoplasmic and nuclear stains were employed in order to bring out all possible details of the anatomy. Haematoxylin and eosin respectively proved to be good nuclear and counter cytoplasmic stains. These gave results with certain optical quality suitable for the employment of high power lenses. A series of figures in parts, and of the cross-sections at various regions of the body has been drawn by using Camera lucida equipment. Graphical reconstruction of the body has been made for the study of various organ systems.

Histopathology of the host tissue was also conducted. Seven days old chickens were utilized for this purpose. The chickens were fed viable eggs of the worms which were embryonated in distilled water with 1% formalin. The chickens were killed at 4 - hrs interval and caeca were

isolated to trace the tissue phase. These were fixed in Bouin's picric acid. Sections were prepared using paraffin microtechnique. Haematoxylin and eosin were used as counter-stains.

Caeca of fowls with natural heterakiasis were also selected for histopathological studies.

Embryonation procedure:

Following media were used for embryonation:

(i)	Formalin	1%
(ii)	Distilled water	
(iii)	Nitric acid	1.5%
(iv)	Potassium dichromate	2.0%
(v)	Hydrochloric acid	10-15%
(vi)	Sulphuric acid	10-12%
(vii)	Acetic acid	0.5%
(viii)	Phenol	0.6%
(ix)	Corrosive sublimate	1-1.5%
(x)	Normal saline	0.75%
(xi)	Alcohol	70%

The eggs were collected by splitting open the adult female and teasing apart the uteri. Coarse particles were washed free of adhering eggs and removed with forceps and pipette. Eggs were then washed in several changes of physiological saline to remove the debris. After counting, the eggs were kept in separate culture dishes with different media. The solutions were changed regularly to maintain the desired concentration. Percentage of embryonation was determined by counting both embryonated and unembryonated eggs in different media.

For the study of post embryonic development, 200 of viable eggs were fed to the laboratory reared chickens. The birds were autopsied after every 4 - hrs. interval and parts of the alimentary canal was examined for developing stages. Various observations were recorded.

Vector borne infection:

To make an assessment on the possibility of vector borne infection, grasshoppers and earthworms were thoroughly investigated. Three set of experiments were planned on the following lines:

(a) Grasshoppers and earthworms collected from the field.

(b) Grasshoppers and earthworms collected around the poultry yards.

(c) Grasshoppers and earthworms reared in the laboratory under infection free condition.

The chickens used in these three major studies were reared and maintained in wire-floored cages under conditions that precluded the possibility of accidental infections with the parasites being studied.

The earthworms collected from the field and around the poultry-yard were rinsed in tap water and transferred to clean soil that had been thoroughly air dried and restored to normal moisture content by sprinkling with water. These worms were transferred through three changes of this clean soil at intervals of approximately 2 days. Worms of the third group were maintained for 1 week between layers of moist filter paper and given a few flakes of rolled oats each day to prevent shrinkage and to induce evacuation of the intestinal contents.

In the first and second set the chickens were directly fed on the grasshoppers and earthworms collected from the fields and around the poultry yards. In the third

set, infection was induced with grasshoppers and earthworms fed on viable eggs and later given to chickens. These chickens were sacrificed after 18 days and number of adult worms recovered on autopsy were recorded. Chickens under controlled conditions were also maintained for comparison.

P A R T - A

M O R P H O L O G Y

GROSS MORPHOLOGY

Heterakis gallinarum is a small creamy white nematode. Size of the worm is variable being influenced by the conditions within the body of the host. The male and female forms are distinct, females are larger and thicker than the males and width of both varies in proportion to their general length. The body in both the sexes is slendrical but tapers gradually towards the extremities. Usually the anterior end of the body is flexed dorsally. The tail end is almost straight in case of female, while in the male the cuticle of the posterior extremity is expanded into a well marked bursa supported by a number of papillae with a chitinous pre-anal sucker.

Average measurements of different parts of an adult male and female taken from ten different specimens are given below:

MALE	Single worm	Average
Total body length	8.94 mm	7.45-9.90 mm
Width at the middle of the body	0.35 mm	0.25-0.40 mm
Distance of the nurve ring from the anterior end	0.30 mm	0.20-0.30 mm

Distance of excretory pore from the anterior end	0.36 mm	0.25-0.45 mm
Length of oesophagus	0.95 mm	0.90-1.15 mm
Length of right spicule	1.23 mm	0.60-2.30 mm
Length of left spicule	0.78 mm	0.52-1.04 mm

FEMALE:

Total body length	11.14 mm	10.20-13.75 mm
Width at the middle of the body	0.38 mm	0.30-0.45 mm
Distance of nerve ring from the anterior end	0.34 mm	0.20-0.40 mm
Distance of excretory pore from the anterior end	0.42 mm	0.30-0.55 mm
Length of oesophagus	1.12 mm	1.0 -1.40 mm
Distance of vulva from the anterior end	5.90 mm	4.60-8.90 mm
Distance of anus to end of tail	1.05 mm	0.85-1.65 mm

The body is covered over by cuticle which is finely striated. The body cuticle is thickened and bears cuticular ridges beginning from 0.04 mm from the anterior end and terminates at a distance of 8.28 mm in male and 9.72 mm in the female.

The mouth is terminal and occupies the centre of the anterior tip. The mouth opening is circular and not

tri-radiate as Clapham (1933) reported earlier. It is surrounded by three almost equal lips, one dorsal and two sub-ventral in position. The lips are highly muscular and their inner margins are denticulate. Chitwood (1932) described the arrangement of the cephalic papillae as the dorsal lip bearing two large duplex papillae, while each of the sub-ventral lips having a large sub-median duplex papillae and smaller simple lateral papillae. These observations were supported by Baker (1936) and the present author also finds a similar condition. Amphids are present on the sub-ventral lips in the lateral position (Fig. 1).

The nerve-ring is present in the anterior part of the oesophagus. The excretory pore opens, slightly behind the nerve ring on the mid-ventral side of the body. The opening is without any specializations of the cuticle and is connected inside with the obliquely placed vesicle like structure.

The alimentary canal is essentially a simple and straight tube extending through the entire length of the body. It begins anteriorly as mouth and terminates in the posterior region on the ventral side through the anus in the female and cloaca in the male. The mouth opens into short and simple buccal cavity (Figs. 2 & 3) at the base of which three denticulate structures have been observed (Fig. 1) which

were described by Baker (1936) as onchi. The buccal cavity follows into a large and muscular oesophagus. The oesophagus is club-shaped and basically of the oxyuroid type (Fig. 5). Baker (1936) described this part of the digestive tract to be formed of a short pharynx and an oesophagus. The present author finds no such differentiation, however, the lumen of the oesophagus which is fairly wide in the anterior part abruptly narrows and continues as such throughout. An oesophago-intestinal valve is present at the junction of oesophagus and intestine. The intestine extends from the pharyngeal bulb as a simple tube and terminates posteriorly into the anus. The cloaca in the male is closely surrounded by the anal papillae (Fig. 4) but in case of female no papillae have been observed. Contrary to this Baker (1936) has reported occurrence of anal papillae in a female. The anal opening of both female and cloaca of male occupy similar position on the ventral side of the body, a short distance from the posterior end.

The female reproductive system is didelphic and amphidelphous in condition (Fig. 6). Both the uteri join and give rise to an elongated structure termed as vagina. The proximal end of the vagina enters a specialized region of the gonoduct known as the ovijector. The ovijector opens

outside through the vulva which lies almost in the middle of the body (Fig. 6).

The male reproductive system consist of a single gonad having a duct which finally opens into the cloaca. It begins with the testes, widens to form the seminal vesicle and communicates further as the ejaculatory duct (Fig. 5). Finally in the posterior region of the body it joins with the digestive tract to form a common passage, the cloaca. The cloaca is located near the rear end of the body. In addition accessory genital organs, caudal papillae and spicules, are also present in this part of the body.

The caudal alae make their appearance as a simple thickened structure a little above the sucker, on the ventral side of the body (Fig. 4). It divides into two large thick lobes at the level of the sucker and converges gradually further and disappear ultimately near the tip of the tail. It was thought earlier that the caudal alae is a continuation of the lateral ridges which maintain a lateral position, the alae arise from the under side of the worm's body.

The expanded lobes of the caudal alae bears the genital papillae. The papillae are arranged as follows: There are 5 papillae close to the sucker region, two are paired

and pedunculate the 5th is sessile and median in position (Fig. 4). Clapham (1933) gave no indication of the presence of any sessile papillae associated with the sucker. Baker (1936), on the other hand reported such a papillae. The presence of six pairs of anal papillae is in accordance with the findings of the former author - 3 pairs are pre-anal, Ist pair is pedunculate and the other is sessile. Following to this are 3 pairs of post anal, the Ist is sessile and the other is pedunculate. Baker (1936) observed an indefinite number of papillae in the tail region, which appears to be incorrect as only 4 pairs of definite papillae could be located by the present author. All are pedunculate, the Ist pair is prominent and the remaining 3 are less so, the last 2 pairs are placed quite adjacent to each other.

The sucker is chitinous in structure and is situated slightly anterior to the cloacal opening of the male (Fig. 5). It has two distinct parts. There is an outer clear area and an inner cupped region.

The spicules are dissimilar in size and shape. The left spicule is shorter than the right one. The shorter spicule is tubular and possess two lateral flanges and the tip has a characteristic twist and often protrudes outside of the body (Fig. 4). The right spicule is also tubular but possess only one lateral flange. The tip is sharp and often hidden inside of the body.

Explanation of figures

Fig. 1. Head of female, *en face* view.

Fig. 2. Dorsal view of anterior end of female.

Fig. 3. Lateral view of anterior end of female.

Fig. 4. Posterior end of male showing sucker caudal papillae and spicules.

Fig. 5. Male, adult worm.

Fig. 6. Female, adult worm.

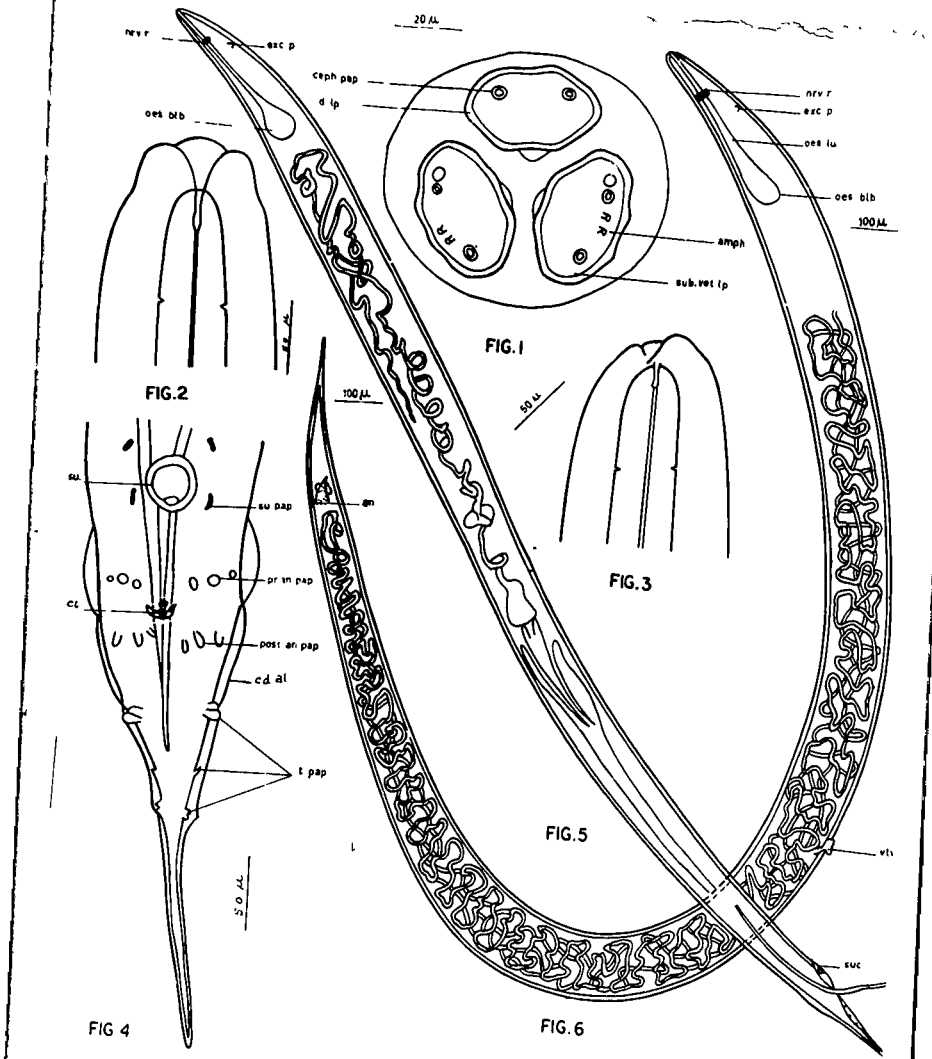
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FIG. 6

HISTOLOGICAL ANATOMY

The histological anatomy of the worm, Heterakis gallinarum was studied in detail.

THE BODY WALL

The body wall of nematodes consist of three distinct components known as cuticula, sub-cuticula and musculature.

Cuticula

It is the outer most covering of the body of nematodes. It covers the body externally and also extends inwards at the mouth, anus and vulva. The cuticle is connected intimately with the underlying sub-cuticula layer (Fig. 9). Chitwood and Chitwood (1950) believed this layer to be a product of hypodermis, although the character of its formation is very controversial. It is completely a non-cellular and highly elastic layer. It appears gelatin like in moist condition. It can be divided into two general

types, the external cuticula which is applied to external coating of the body and the internal cuticle which includes the parts of the cuticle covering the oesophagus, rectum, cloaca and vagina. The external cuticle is supposed to constitute the main body cuticle in this nematode. It covers all the apertures, such as mouth, anus, cloaca and vulva to become continuous with the cuticular lining of all the cavities into which these apertures open. The body cuticle presents a number of modifications. These modifications of cuticular structures are made up of superficial layers of the cuticle in most of the cases. The various external structures, such as lateral ridges, the prominent caudal alae (Fig. 8), the pre-anal sucker (Fig. 14) of the male are also the modifications of various layers of the cuticle. Baker (1936) was also of the opinion that these external structures do not arise as out foldings of the body wall but are the modifications of the cuticle.

Von Siebold (1848) while studying the cuticle of Ascaris lumbricoides, observed that it is not single layered as apparently seems to be, but is made up of complex layers. De Man (1886) described almost a similar pattern to occur in Enoplus communis. Bastian (1886) for the first time came forward with the view that the cuticle of nematode was

divisible into five distinct layers. He also gave the description and position of various layers using his own terminology for various layers. The cuticle of Ascaris and Oxyuris was studied in detail by Goldschmidt (1905) and Martini (1912) respectively. They too concluded that the cuticle is made up of complex layers and the former author elaborated at least 9 layers in the cuticle of Ascaris. Ansari and Basir (1964), however, could locate only eight layers in Setaria cervi. But Baker (1936) observed only two layers in the cuticle of Heterakis gallinarum. He also gave no description of any sort which reflects that his approach has been of a very superficial nature. The present author observed five distinct layers in this worm (Fig. 10 & 11). These layers from outside inwards are as follows:

External cuticular layer Internal cuticular layer	Cortex layer
Fibrillar layer Matrix layer Basal lamella	Matrix layer

The external cuticular layer is much denser than the internal cuticular layer and the matrix layer. The internal cuticular layer does not appear to be an actual entity since

it is continuous with and often quite similar in consistency to the matrix layer from which it is grossly separated by the fibrillar layer. The latter has the appearance of a condensed solid mass formed of a closely woven net work of fibrils. The matrix layer is commonly known as 'homogeneous layer'. It appears as a finely alveolar or spongy mass. The basal lamella is a very thin layer and looks like a striated membrane. The average thickness of the layers is given below.

External cuticular layer	1.0 μ
Internal cuticular layer	1.50 μ
Fibrillar layer	1.0 μ
Matrix layer	7.0 μ
Basal lamella	1.0 μ

The various layers of the cuticula as described are not always to be found throughout the body of the nematode, and the relative thickness of the different layers may change to a remarkable degree. Certain specialized regions such as the vulva, anus, excretory-pore and cloaca have only the cuticular layer.

There are two lateral ridges present on either side of the body (Fig. 9) which make their appearance at about

0.04 mm from the anterior end. It terminates posteriorly a little away from the caudal alae. Structurally both the lateral ridges are similar throughout, well developed in the pre-equatorial region and gradually reduced posteriorly. It is composed of an outer cortex layer, which encloses within it a deeply staining V-shaped region (Fig. 12). The base of the V is outwardly directed and the two limbs tapering to a point inside. Most of the space covered over by the base of the V- and its two limbs is occupied by a grannular substance. The basal lamella of the general body cuticle forms the innermost boundary layer for the grannular substance. Baker (1936) has also given description of the lateral ridges, but his observation that the basal lamella within the regions of the lateral ridges bifurcates and encloses a similar grannular region derived from the grannular mass could not be verified in the present study. However, the author agrees with the idea presented by Baker (1936) that the lateral ridges are differentiated into 3 regions, but he could not name those regions and on the contrary suggested about the darkly staining regions as a deposit of special material. The author is of the opinion that the regions are actually elaborated layers of the cuticle, which take part in the formation of the ridges. Various layers taking part, are the outer and inner cortical layers enclosing

the more elaborated matrix layer. The matrix layer is further differentiated, the darkly staining region is the fibrillar layer composed of condensed fibrils and following to this is the grannular region which is actually the spongy matrix layer. Fibre layers are absent and basal lammella forms the boundary layer.

Sub-cuticula

The sub-cuticula is the middle layer of the body wall lying in between the cuticula and the somatic musculature (Fig. 9). It is also sometimes referred to as hypodermis. It is a syncytial layer madeup of fine sheet of protoplasm containing grannular substance. The sub-cuticula is very much reduced in the anterior and rear end of the body so that the muscles come in direct contact with the cuticle. This layer bulges out into the pseudocoel at four places in the form of four longitudinal chards dividing the column of the body muscles into four sectors. The longitudinal lines or bands are very narrow and indistinct except the lateral chards which are well developed and easily seen in cross-section.

Eberth (1860) reported the presence of large number of nuclei in the sub-cuticula layer of Heterakis vesicularis.

Baker (1936) observed no such nuclei in the sub-cuticular region in case of Heterakis gallinae. But the present author has located nuclei in the region of the chords only.

Each of the longitudinal chords begins as a small thickened structure in the form of a grannular mass from the anterior end of the body. Soon the inner bulgings increase in height and the four chords get separated from one another near the middle region of the oesophagus (Fig.21). Marked differences in the size of the chords has been observed from the middle region of the oesophagus onwards. The lateral chords become comparatively larger, broader and conspicuous, while the dorsal and ventral chords assume quite insignificant shape between their respective groups of muscle cells.

A carefull study revealed that the size and position of the lateral chords varied to a great degree. The reason for this is not known. Among the earlier workers, it was Bastian (1866) who gave certain account about the lateral chords. The author described them as intra-muscular derivatives of the sub-cuticula. He also mentioned a fibrous frame-work arising out of the central proximal region of these chords which after dividing runs towards the distal margin on either side of the excretory tube.

But no such condition was observed in the present worm.

The tissue mass of the chord is syncytial, but the cytoplasm around the nuclei takes up a darker stain as compared to the surrounding mass. The lateral chords are broad and low in females, but narrow and high in the male. These remain simple and almost alike throughout the body length, except at the regions of the nerve-ring (Fig. 32), excretory pore (Fig. 25 & 26), anus (Fig. 17) and vulva (Fig. 43) where these undergo some modification. The two chords extend inwards so as to merge with each other at the level of the nerve-ring, enclosing within their mass the oesophagus and the nerve ring. In the oesophageal region the lateral chords bulge freely while opposite the gonads these become very much flattened. The present author agrees with Baker (1936) that the lateral chords expand considerably in the posterior region of the body.

Baker (1936) regarded the fibrillar frame-work of the lateral chords behaving like a line of demarcation between the various chains of primitive cellular component making the chords. The so-called cell walls thus formed in the form of darkly staining lines, divide the lateral chords into three fields (Fig. 13). The width of these

three fields is not equal. The lateral field is considerably extended at its free end and the excretory tube is usually carried within this region. The dorsal and ventral field of the lateral chords are also expanded.

It appears that the dorsal and ventral fields of the lateral chords arise and expand from and beneath the adjacent muscles layers. The present author agree with Baker (1936) that through most of the length the nucleation of these three fields is very definite. Nuclei are also present in the lateral field of the lateral chords, but at much greater and more regular intervals than do the adjacent fields.

The dorsal chord is the smallestand most inconspicuous of all the longitudinal lines. It is like thin strand of protoplasm, placed at a little distance towards the posterior side to the level of the lateral chords (Fig. 15). The dorsal chord never extends along the entire length of the body and keeps direct contact with the somatic muscles at the two ends. In the anterior region, at the level of the nerve-ring the chord sends out tissues which connect the lateral chords on either side and partly covers the nerve-ring. Further in its course towards the posterior

side, the dorsal chord, retaining the same width, traverses a fairly long distance and gradually gets reduced and becomes very insignificant. The chord carries the dorsal longitudinal nerve and receives the innervating processes from the somatic muscle cells arising from both the sides of the dorsal half of the body. The chord appears to be made up from a single series of hypodermal elements but cell walls could not be distinguished. Small nuclei are present at different levels.

The ventral chord appears at the level of origin of the dorsal chord, but unlike the latter it continues upto the end of the tail. Soon its height increases and projects far away into the body cavity, while its base which is in contact with the cuticle remains compressed by somatic muscles. The ventral chord is modified in the region of the excretory pore, vulva and anus. The excretory pore and vulva open outside through this chord (Fig. 25, & 43). At the position of anal aperture the chord is pushed aside and the anus opens outside (Fig. 17). At the level of the nerve-ring (Fig. 32) the ventral chord along with the terminal portions of the excretory apparatus becomes closely connected with the lateral chords on either side. From this region onward the band retain the same height and width. Its histological details are similar to those

of the dorsal chord, even the number of nuclei and their arrangement remains the same.

Musculature

The musculature is divisible into two types i.e. the somatic musculature and the specialized musculature. The somatic musculature is the general muscular layer of the body wall of nematode and is composed of a single layer of more or less spindle shaped cells attached to the sub-cuticula throughout their length. Baker (1936) contrary to this view, believed that the muscle cells are directly attached to the cuticula layer of the body wall. These groups of cells lying parallel are separated by the longitudinal thickenings. Due to the presence of chords, this layer of muscle cells is divided into a number of sectors. The muscle cells in each sector usually act as a unit during body movement.

Heterakis gallinarum has polymyarian and coelomyarian type of musculature i.e. there are many muscle cells in one sector (Fig. 9) and the fibrillar portion of the cell bear distally a groove making the sarcoplasmic part to dip down into and between the contractile layers present on either side of the cell (Fig. 7). The cells lie parallel to one

another and are divided into four fields by four longitudinal lines, two sub-dorsal and two sub-ventral in position. These muscles appear anteriorly in the form of four tissue masses, one in each sector, at the anterior most end of the body. Proceeding a few micra posteriorly, the tissue masses are pushed towards the periphery. This is preceeded by the appearance of the fibrillar portion of the muscle cells that get closely applied to the cuticula. The fibrillar part of the cells is slightly raised distally. From this point onwares upto the level of the nerve-ring, the cell becomes higher and project more or less like knobs into the body cavity. At this level fibrillar portion of the muscles never appeared grooved. Further onwards the normal somatic muscle cells increase in height and a little behind the nerve-ring assume the typical form of somatic musculature.

A detailed study of the individual muscle cell reveal these to be made up of a fibrillar region and a sarcoplasmic region. The former close to the hypodermis layer is comprised of a pile of ribbons or bands of homogeneous contractile substance alternating with a non-contractile one containing the supporting fibrils, and the latter is just a protoplasmic mass having a net work of contractile fibrils which also retains the nucleus (Fig. 7).

The fibrillar part of the muscle has the shape of a V or U with the notch varying in depth. The fibrillar portion of the muscle cells do not end abruptly but tapers down to a fine point at either ends. Baker (1936) explained that these fine ends appear as small muscle cells wedged in between the larger muscle cells. Such a condition was not observed in the present study. The ratio between width and length as given by him was almost 100 times longer than broad, but the present author found the basal width of a cell as about 0.005 mm, while the length was found to range as high as 0.600 mm in average.

There is a unique tendency of the muscle cells to converge towards the median line at the ventral half of the body. These also give out processes towards the motor nerves. Baker (1936) gave similar observation and explained that not only the prolonged processes of the muscle cells run to the median nerves but the muscle cells themselves are lined together by the former structure.

In case of males the musculature almost remains the same with a little difference that the muscle bands hang deep into the body cavity occupying most of the space.

THE BODY CAVITY

Earlier the body cavity of nematodes has been compared to a segmentation cavity and to a coelome, but Rauther (1909) pointed out that neither comparison is possible. The body cavity in nematodes is not lined by a mesodermal layer on its either side and so it is called as pseudocoelome. In case of Heterakis gallinarum it extends almost the entire length of the body, except the region of the oesophagus and the portion behind the anus where it is almost obliterated by the massive growth of the connective tissue. In the intestinal region, the greater part of the body cavity is quite narrow because of the close association of the gut to the body cell and the presence of the reproductive organs.

The pseudocoelomic fluid

The pseudocoelome is fluid filled space. The fluid is thick and contains dissolved substance in the form of globules. When the worm is treated with a fixative, the fluid becomes sedimented and gets deposited. In cross-section these are seen as thick spongy mass of granules with vesicular cavities in between them. Those regions of the

spongy mass which acquire the form of threads or lamellae take up haematoxylin stain deeply. This has been found localized in specified parts of the body, usually in the oesophageal region and the region of the hind gut. No nuclei have been found in this region.

The exact nature of the pseudocoelomic fluid is not very well known and needs a thorough investigation. Ansari and Basir (1964) pointed out two possibilities regarding the nature of the pseudocoelomic fluid: (1) a physiological body fluid coagulable by fixatives or (2) a mesenchymatous tissue of low organization. It is extremely difficult to determine validity of either of the above conditions due to the lack of proper tests which could enable a microscopical differentiation between a coagulum and lowly organized tissue.

Connective tissue

It is in the form of a net work of nucleated strands of fibrous tissue which occupies the whole space of the inner surface between the oesophagus and body wall. Generally the direction of the tissue is from the inner muscular layer towards the oesophagus. It takes the form of support or

mesentric tissue round the oesophagus and continues anteriorly to encircle the buccal region. The oesophagus is also surrounded by the strands of fibres which are attached with the muscle cells and various chords. Most of the organs present in the body cavity are sheathed by a very thin covering of connective tissue fibres. Large sized nuclei were found scattered in the general mass of the connective tissue. The region of the connective tissue takes a deeper stain or eosin. At the region of the nerve-ring the connective tissue is not in abundance because of the presence of numerous ganglionic cells (Fig. 32). The tissue decreases gradually at the beginning of the intestine. In the posterior most part of the anal region, the various chords project inwards to such an extent that it was found impossible to distinguish this tissue from the connective tissue.

Explanation of figures

Fig. 7. T.S. of a typical somatic cell.

Fig. 8. T.S. passing through the caudal alae.

Fig. 9. T.S. passing through the region of somato-intestinal muscles.

Fig. 10. Diagrammatic representation of the layers of cuticle.

Fig. 11. T.S. through the cuticle of the body.

Fig. 12. T.S. through the dorsal ridge.

Fig. 13. T.S. through the lateral chord showing the partition canal.

Fig. 14. T.S. passing through the sucker.

Fig. 15. T.S. passing through the region of oesophagus showing dorsal chord, lateral chord and ventral chord.

Fig. 16. T.S. passing through the region of the intestine.

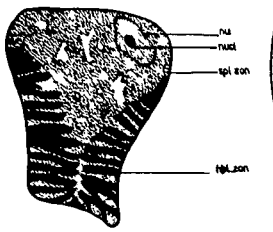


FIG 7

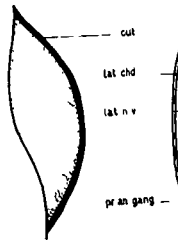


FIG 8

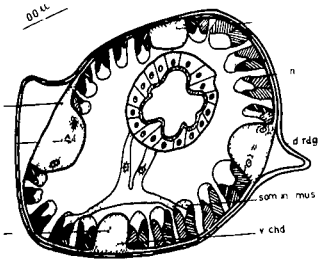


FIG 9

FIG 10

FIG 11

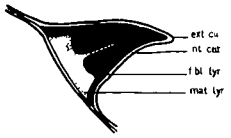


FIG 12

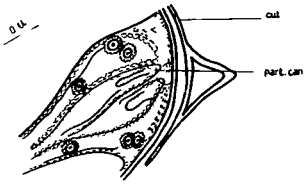


FIG 13

FIG 14

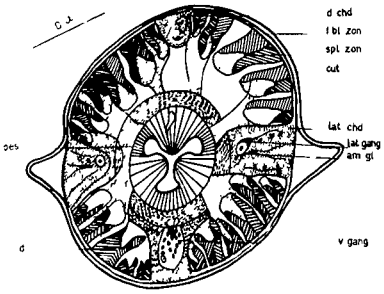


FIG 15

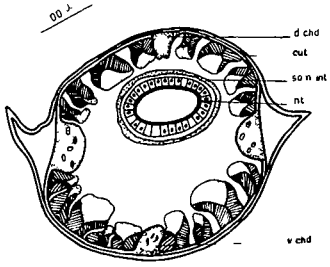


FIG 16

DIGESTIVE SYSTEM

The digestive system in Heterakis gallinarum comprized of a simple and straight tube extending throughout the body from anterior mouth opening to terminate in the posterior part on the ventral side of the body. The mouth leads through a short stoma into a muscular pharynx. It follows further as a club-shaped oesophagus and communicates into the intestine via a valve. The intestine which is in the form of a long narrow straight tube open through the intestino-rectal valve into the rectum. In the female the rectum opens to the exterior by a ventrally situated anus while in the male the reproductive ducts unite with it to form a cloaca which ultimately opens to the exterior on the ventral side as the common ano-genital aperture.

Mouth and cephalic structures

The mouth is terminal and occupies the centre of the anterior tip. The opening is rounded and not tri-radiate as reported by Clapham (1933). It is surrounded by three large almost equal sized lips. The lips are named according to their position, one dorsal and two sub-ventral. Two large duplex papillae are present on the dorsal lip, while each of

the sub-ventral lip has a large sub-median duplex papillae and smaller simple lateral papillae and amphids (Fig. 1).

Clapham (1933) failed to locate the presence of teeth associated with the lips, but Uribe (1922) and Baker (1936) reported such a structure. According to the latter author each of the lip being provided with a pair of teeth, a set of large anterior teeth and another set of smaller teeth placed posteriorly. The present author has found these teeth as spine like projection placed on the inner surface of the lips. Since these teeth as explained by Baker (1936) are incapable of making independent movement and also their position in relation to each other are not always constant, the present author has every doubt about the validity of these structures as teeth. Baker, himself took these structures as organs for adhesion and never believed these could function as chewing structure (Fig. 1).

The movement of the lips is brought about by the attached muscles (Fig. 20). The mouth cavity is opened by the backward pull of the muscles attached inside the outer wall of the lips. In this way the lips are drawn apart making the inner surface of the lips more and more convex. The contraction of the retractor muscles of the lips secure firm grip against the intestinal wall of the host.

Buccal cavity

The mouth leads into a short buccal cavity or stoma. It connects the mouth opening with the pharynx. The lumen is circular and quite narrow. The wall of the stoma is devoid of any armament.

Oesophagus

The lumen of the oesophagus shows quite a uniform tri-radiate symmetry in cross-section. Normally one of the rays always points towards the e ventral side, the remaining two being sub-dorsal in position (Fig. 21). Circular enlargements of the lumen are present at the ends of three rays slightly anterior to the oesophageal bulb (Fig. 15). These have been named by Baker (1936) as the circular canal with the wall supposed to be made of homogeneous secretary products. Immink (1924) while studying Strongylus oedentatus has shown that the wall of the lumen is not of pure chitin but of an albiminoid substance.

The external covering of the oesophagus, a structureless layer, 'tunica propria' covers it throughout its length. The tunica propria though seems to be cuticular, but there exist a considerable difference of opinion regarding its origin

and nature. It is regarded as secretion product of the muscle cells, while others including Martini (1908) differ with this view and observed that marginal nuclei are responsible for its development. Chitwood (1930) also agreed with this idea as more logical and convincing.

The oesophagus is a syncytial structure. A large number of different types of tissues, nuclei and a variety of other muscle bands, nerve-cells and many other cells of unknown nature together from the oesophagus. The protoplasm of each of the oesophageal glands generally retain its identity and nuclei of the muscles are placed as to have no doubt that each belong to a specific fibre (Fig. 21).

Two types of fibres consititute the musculature of the oesophagus. They are known as marginal muscles fibres and radial muscle fibres (Fig. 21). These could be easily recognised by their points of attachment. The small group of fibres which connects the distal ends of the rays to the periphery are known as marginal muscles. These muscles are inserted in the thick knob-like structure present at the end points of each ray which are specially meant for this purpose. The protoplasmic strands associated with these fibres are sarcoplasmic. The marginal muscles are highly developed in this region and the lumen of the oesophagus is kept in its position by these muscles.

The other type of muscles namely, the radial muscles are more distinct and quite numerous as compared to the marginal fibres. These muscles are inserted at one end on the sides of the oesophageal rays and the other end is inserted to the external wall of the oesophagus.

The oesophageal bulb

The lumen of the oesophagus undergoes a considerable change at the point where the oesophageal bulb arises. When the lumen is traced deep into the bulb it was observed that the rays increase considerably in depth but the normal tri-radiate symmetry is maintained as shown by the rest of the lumen of the oesophagus. The lumen enlarges considerably in the centre of the bulb and at the middle there are present three almost knob-like structures which arise from the wall of the lumen and found to have a prominent rough inner surface which extends into the lumen. These structures were described by Baker (1936) as 'roller' and were considered to be associated with valvular apparatus. Their presence in the oesophageal bulb provides a strong plea that these are an accessary part of the oesophageal falve. But their variable position observed by Baker (1936) in different whole mount preparations presents logical function as structures concerned with regulation of the movement of food from anterior to

posterior.

The bulb is highly muscular and possesscs large and important glandular structures. Here also the marginal and radial muscles are present, which likewise operate for the support and for the enlargement of the oesophagus. The muscles are more developed and take an oblique position anteriorly and posteriorly. The oesophageal glands lie in between the large muscle fibres found between each rays of the lumen.

The oesophageal glands

There are three oesophageal glands as three gland nuclei could be distinguished. One nucleus lying in each of the three areas divided by rays of the lumen. These gland cells, one dorsal and two ventro-lateral are fused in the posterior part of the oesophageal bulb. These appear simultaneously but open at different levels directly into the lumen of the oesophagus by means of ducts. The openings of the ventro-lateral glands were located within the oesophageal bulb.

The glands take their position in between the muscle fibres because of their lobed structure and numerous glandular

remifications. A longitudinal duct runs in each gland which appears to be devoid of any cuticular lining. The cytoplasm of this region shows a structureless outer memberane and an inner granular portion. These granules are large but they are few in number so as to leave many spaces within the glands.

The function and significance of these glands is far from certain . However, their position and opening into the lumen of the oesophagus would no doubt, suggest some digestive function.

Oesophago-intestinal valve

Most of the nematodes possess a valve at the point where the oesophagus meets the intestine. A valve is also present in the case of Heterakis gallinarum, as a laterally flattened structure at the junction of the oesophagus and intestine (Fig. 19). A cuticular coating is present at the inner lining of the valve which makes it a continuous structure with that of the lumen of the oesophagus. The valvular apparatus is placed in the cavity formed by the anterior end of the intestinal wall which bends inwards and backwards in the form of funnel (Fig. 23). It appears as a small circular channel. A number of changes have been observed in the lumen of the oesophagus, before the formation

of the valve. From the centre of the oesophageal bulb the lumen narrows considerably towards the posterior end, one of the sub-dorsal rays begin to shorten and the lumen appears like a ventral slit with the dorsal rays disappearing at this point. Gradually an increase in the radial musculature was also observed in the lumen of the oesophagus which were found attached with the sides of the lumen. The opening and closing of the wall is brought about by sphinctor muscles which are in the form of circular bands of fibres surrounding the valve.

Intestine

The intestine of nematodes is just like a straight tube, the wall of which is composed of epithelial cells. Its gross morphology does not differ much from other nematodes.

A single layer of tall and hexagonal columnar epithelial cells form the wall of the intestine, which generally remains as such throughout its entirelength (Fig. 24). It may be divided into three different regions. The anterior or ventricular region, mid region or intestine proper and the posterior part or the pre-rectal region.

The ventricular and pre-rectal regions commonly differ from the mid-region or the intestine proper in the height of

of the cells and shape of the lumen. Usually there is also some difference in the type of cell inclusions present in these regions. The cardiac swelling is present which has been formed by the dilatation of the anterior region of the intestine. These cells are 2-3 times as high as wide. The lumen of this region is laterally flattened and irregular in out line. The cells of the mid-region are cuboidal and the lumen is quite regular, while in the pre-rectal region the smoothness is lost again. The cells give rise to irregular extensions and once again the lumen assumes more or less tri-radiate shape.

The author agrees with Baker (1936) that in case of Heterakis gallinarum the walls of the intestinal lumen are lined with a bacillary layer or 'Stabchensaum', but sometimes a distinct sub-bacillary layer or 'Deckschicht' is also apparent. The bacillary layer is considered to be the characteristic feature of the inner surface of the intestinal cells of nematodes and appears to be closely packed, short rod-like structures which project into the lumen.

The protoplasm of the epithelial cells, is divisible into distinct zones. The protoplasmic zone of each cell

containing a nucleus, lies between the sub-bacillary layer and the basal lamella which happened to be the outermost layer of the cell (Fig. 22). The cell boundaries and various layers are distinct in the anterior region but less and less so posteriorly. The details of the different layers are as follows:

Bacillary layer:

The bacillary layer consists of an internal hyaline border appearing to be made up of fine rods or cilia on the free edges of epithelial cells. The rods generally appear to be fused together and thus forming an almost solid membrane. At certain places the rods were found to retain their individuality. A delicate fibril arise from the base of each rod and extends into the cytoplasm of the surrounding cells through the sub-bacillary layer. The cardiac part of the intestine having epithelial cells are provided with a high 'Stabchensaum'. It is often more than one third of the total height of the cells. A gradual decrease in 'Stabchensaum', was found to occur in the pre-rectal region where it becomes very low.

It was found very difficult to determine the nature and significance of this layer. Some earlier workers

believed that it develops from some material from the intestinal contents. Jagerskioeld (1894) found that 'Stabchensaum' consists of long separate rod like structures which project into the lumen. Looss (1905) referred 'Stabchensaum' as a cuticular layer. Quak (1913) after making a detailed and very careful examination of the intestinal cells concluded that the individual rods as described by Jagerskioeld are in the form of longitudinal series of alveoli and that the intra-alveolar substances from the intestinal contents hold them up together in their position. Hetherington (1932) believed that sub-bacillary layer consists of fine granules which appeared to be connected with each other and with the rods which form the bacillary layer. Muller (1929), while working with Ascaris lumbricoides gave his opinion that the bacillary and sub-bacillary layers are not peculiar to nematodes, but occurs in the intestine of various groups of worms as well as in arthropods and vertebrates.

Sub-bacillary layer

This layer was named by Baker (1936) as reticular layer. It stains very sharply with iron haematoxylin and and appears like a dense mass of reticulum or a layer of

granules. Due to the specialized character of this layer, it is also sometimes termed as 'Deckschicht'. This granular character of the sub-bacillary layer supports the hypothesis that the 'Stabchensaum' has been originated from the cilia like structures as the position of sub-bacillary layer suggests, which correspond to the layers of the basal granules in ciliated epithelium. However, Baker identified the sub-bacillary layer as reticular layer.

Protoplasmic zone:

The presence of protoplasmic zone (Fig. 22), seems to be of no special significance in nemic study. The nature of the protoplasm in this zone varies. Sometimes it appears to be alveolar and at other times it is granular. It forms comparatively dense zone right beneath the sub-bacillary layer. Schneider (1902), found it fit to call as 'nutritorishe zone' or nutritional zone. A net-work of plasma strand is present in the granular endoplasm. These granules are supposed to be the digested food particles which the parasite collected during the course of its absorption. Baker (1936) considered, this part of the intestinal cell quite different and called it as the trabaculae. The trabaculae appeared as fine fibrillar strands which help in

the stimulation of neuromotor apparatus. He got the support in this respect from observations made by Muller (1929) in case of Ascaris lumbricoides. Whereas Looss (1905) described such structures in hookworm as 'brown or black pigment' derived from the colouring matter of the blood. Since Heterakis gallinarum feeds on the caecal contents which also contain blood pigment etc. The present author agrees with the opinion expressed by Looss and believes that these granules are no doubt digested food particles. The protoplasm of the cardiac part of the cells contains large vacoules or alveoli. The external part of the cell has a single nucleus with a large nucleolus. The presence of more than one nucleus is not very common and is rarely found. A dense region is again formed at the external most part of the protoplasmic zone which is sometimes termed as 'basoplasm'. It appears that a thin ectoplasmic membrane externally covers the whole protoplasmic mass.

Basal lamella:

A homogenous layer termed as basal lamella (Fig. 22) is present in immediate contact with external cell surface. Apparently this layer is a supporting structure and a secretion product of the intestinal epithelium. There is

no doubt that it acts as a protective sheath for the intestine.

Somato-intestinal muscles are present in the pre-rectal region of the intestine (Fig. 9 & 16). The position and insertion of these muscles suggest that these should be supportive in function.

Intestino-rectal valve

As the oesophago-intestinal valve is present at the place where it opens into the intestine, the intestine also forms an intestino-rectal valve at the place where it communicates with the rectum (Fig. 67). It is a simple structure and is formed by the extension of terminal portion of the intestine which narrows and projects into the lumen of the rectum in the form of a valve. The intestine becomes swollen just before the formation of this valve and at this region its epithelial cells are much smaller and very compactly arranged. The action of the valve is controlled by a sphincter muscles which encircles it completely.

The posterior gut

The last portion of the alimentary tract is known as the posterior gut. It lies beyond the mesenteron or the

intestine terminating into an aperture called the anus which opens outside the body in the tail region on the ventral side. It is always internally lined by the cuticular layer and formed as a proctodeum. The cuticular layer was found in continuation with the external cuticular layer of the body. It comprised of the following parts.

(i) Rectum
(ii) Rectal glands
(iii) Cloaca

Rectum:

The rectum is more or less a short flattened irregular tube. It is formed by the invagination of the cuticular covering of the body of nematodes. The anterior region of the rectum extends over and covers the posterior tip of the intestine thus, enclosing the intestino-rectal valve as well. The rectal lumen is dorso-ventrally compressed at its beginning. It assumes an irregular shape giving out extension in all the directions. It becomes slit-like in the last region and finally opens outside through the anus which is present on the ventral side.

In the case of male, a common ano-genital passage, the cloaca is present. The rectum thus differs a little

from the rectum or anus which is present in the female. In male also it is in the form of a short and flattened tube like that of the female cuticular lining muscle supply and other structures (Fig. 67).

The musculature of the rectum has already been described in detail. The dorsal wall of the rectum elevated by the depressor ani which helps the material to be drawn into the rectal cavity. The dilator muscles on the other hand help in elevation of the posterior tip, and thus defaecation is brought about. No circular muscles were found to occur around the rectum and the phenomenon of defaecation is accompanied by the pressure.

Rectal gland:

The rectal glands are present at the point where intestine and the rectum meet each other. These are three in number but each occupy different locations (Fig. 18). One of these glands is located dorsal to the alimentary canal and the other two occupy latero-ventral positions. The ventro-lateral rectal glands has its opening slightly more anterior than the other two.

In the case of male also the rectal glands (Fig. 67) occupy almost similar position as those of the female. But

here the situation is rather complicated, due to the presence of the reproductive structures which are to be found in this region. The dorsal rectal gland is larger in the case of male than in the corresponding gland of the female. In the case of male it consists of three cells while in female it is composed of only one cell. As in the case of female, the dorsal rectal gland of the male does not discharge its contents into the digestive tract at the same level as the other two rectal glands. But the dorsal gland opens slightly more anterior. In the case of male, the latero-ventral rectal glands, like those of female, consist of a single cell and each gland contains a prominent single nucleus. A close association between the latero-ventral rectal glands and the lateral chords has been observed. At the same point small ducts have also been observed arising from the rectal glands at places where it joins the alimentary canal.

Cloaca:

The cloaca is found only in the males. The vas deferens joins the rectum just posterior to the intestino-rectal valve from the ventral side. Its passage is very short and internally lined with cuticle and covered over externally by a cellular layer. Certain structures of the

male reproductive system, such as the spicules which are associated with it will be described under the reproductive system. The cloaca terminates posteriorly into a ventrally situated cloacal orifice (Fig. 72).

Explanation of figures

Fig. 17. T.S. passing through the anal region of the body.

Fig. 18. T.S. passing through the rectal glands.

Fig. 19. T.S. passing through the oesophago-intestinal valve.

Fig. 20. Longitudinal section showing the mouth cavity and the musculature of the lips.

Fig. 21. T.S. passing through the region of oesophagus showing marginal muscles and radial muscles.

Fig. 22. T.S. of an epithelial cell of the intestine.

Fig. 23. Reconstruction of the oesophago-intestinal valve.

Fig. 24. T.S. of intestinal lumen showing the bacillary layer.

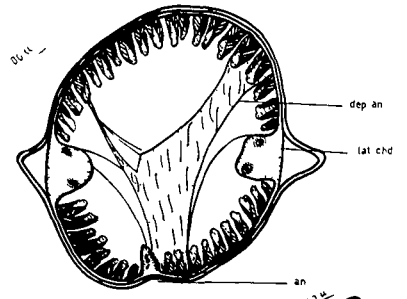


FIG 17

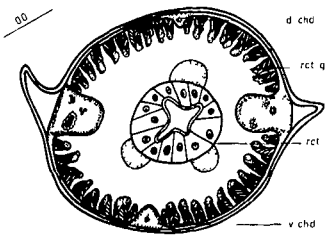


FIG 18

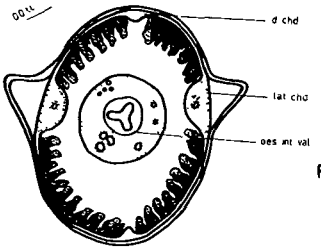


FIG 19

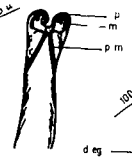


FIG 20

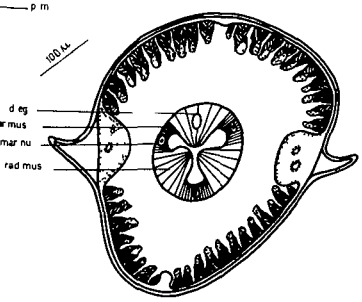


FIG 21

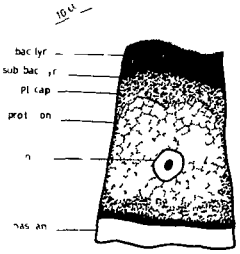


FIG 22

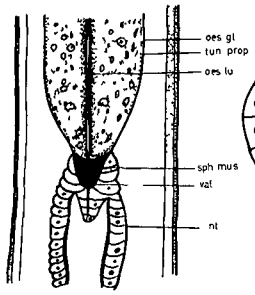


FIG 23

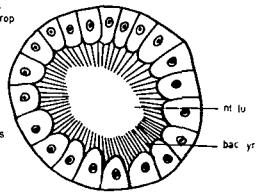


FIG 24

EXCRETORY SYSTEM

Preliminary studies on the excretory system of Heterakis gallinarum has been conducted in order to make more detailed observation than presented by earlier workers. The system appears to be an H-type, the parts comprised are--- excretory pore, terminal excretory duct, the excretory sinus and a pair of longitudinal excretory canal (Fig. 31).

The excretory system opens outside the body in the form of a pore (Fig. 25) and internally leads into a terminal duct until it merges with the excretory sinus (Fig. 26). This sinus gives out two transverse excretory ducts which open in the lateral excretory canals present in the lateral chords. The lateral excretory canals extend only for short distance anterior to the place where the transverse excretory canal joins.

The excretory pore (Fig. 29) is located, in both male and female near the anterior end of the body shortly behind the nerve ring. It takes its position on the mid-ventral part of the body. The pore lacks cuticular specializations or lips. Internally it leads into the terminal duct which

passes through the tissues of the ventral chords, first, in an antero-dorsal direction, then leads backwards and opens into the excretory sinus. Baker (1936) described the excretory tubes as circular in shape with a definite outline. So far the lumen of the duct is concerned, it has been found to be irregular. The wall of the duct is formed by the hypodermal tissue which is internally lined with the cuticle, the latter being continuous with the body cuticle through the excretory pore. The point where the terminal duct joins the excretory sinus, fine bands of fibrous tissue in the form of sphincter muscles have been observed. It is supposed that this portion of the duct functions as a valve and thus controls the flow of excretory waste outside. However, Baker (1936) did not mention such a structure, perhaps the author missed to locate it during observation.

The excretory sinus

The excretory sinus (Fig. 26) also sometimes termed as excretory vesicle or excretory bulb is considered as the storage structure. The lumen of the sinus does not have a definite shape in all cases but is variable. The valve is made up of alveolate or granular cytoplasm. A large nucleus is also present which is known as sinus nucleus. The sinus

is present within the tissue of the bridge and on each side it gives out transverse excretory ducts (Fig. 25) which in their turn open into the lateral excretory canal of respective side (Fig. 28).

The excretory bridge

It is formed by the extension and fusion of the lateral and ventral chords (Fig. 30). The junction of anterior transverse excretory tube occurs within the bridge. It is not certain as how far the lateral chords take part in the formation of excretory bridge but sometimes the latter organs show the signs of fibrous elements in their outer parts. A large nucleus is present at the point where the lateral excretory tube come together which is in close contact with the parts on the ventral side. There are extensions, which arise from the lateral chords and thus join the excretory bridge. It then rapidly divides along the median line to become continuous with the wall of the tubes. The excretory tubes enter the lateral chords through the central field and from their distal edge run far backward in the body. It is suggested that the bridge serves to hold and give support to the excretory canal, excretory sinus and terminal excretory duct.

The lateral excretory canals are present within the lateral chords near their inner margins (Fig. 27). They are well developed and end as blind tubes on either side. Ampulla-like structures could not be observed at the terminal end of the excretory canals. The lumen of the canal is moderate with a fairly regular lining.

From the anatomical stand point, the close association of the excretory tube with the V-shaped area of the chord is very significant. It was Goldschmidt who expressed the view that the adjacent area of the lateral chords which hold in it the excretory canals are specialized structures and represent a sort of true-kidney of nematodes. In case of Heterakis gallinarum, such area of jelly-like matrix with fibrous elements distinct from the rest of the chord extending from the base of the lateral lines towards the excretory duct have been observed. Baker (1936) also, reported such a structure but exprcssed doubt about its true nature. Goldschmidt's idea, seemed to be quite reasonable, but in the present study any intracellular connection between the granular area of the chord with lateral excretory canal could not be observed.

Explanation of figures

Fig. 25. T.S. passing through the region of excretory bridge and lateral excretory canal.

Fig. 26. T.S. passing through the excretory pore, the terminal excretory duct and the excretory sinus.

Fig. 27. T.S. passing through the lateral chord showing the lateral excretory sinus.

Fig. 28. T.S. passing through the lateral excretory canals.

Fig. 29. T.S. passing through the excretory pore.

Fig. 30. T.S. passing through the excretory sinus.

Fig. 31. Diagrammatic reconstruction of the excretory system.

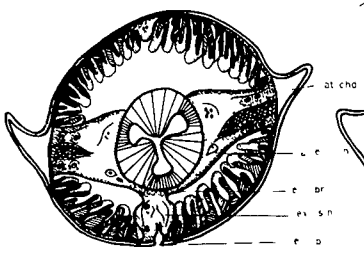


FIG 25

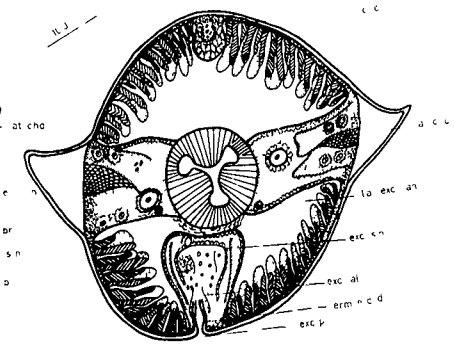


FIG 26

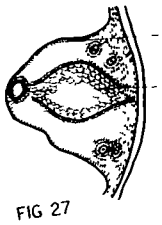


FIG 27

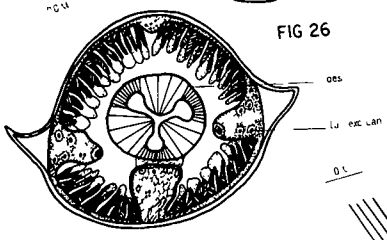


FIG 28

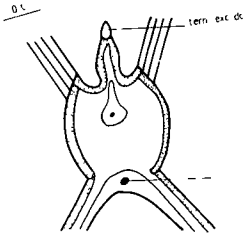


FIG 31

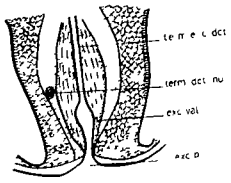


FIG 29

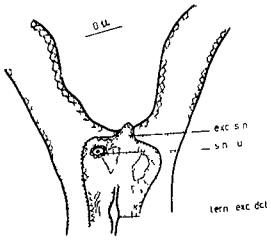


FIG 30

NERVOUS SYSTEM

Basic knowledge regarding the nervous system of nematodes is very limited and fragmentary. Whatever, knowledge regarding this system is available is based on the results and observation made by Hesse (1892), Apathy (1894), Deineka (1908) and Goldschmidt (1908) during the study of nervous system of Ascaris lumbricoides and Ascaris megalocephala.

Many known methods after repeated trials, have been employed to demonstrate the nervous system in Heterakis gallinarum. Like most nematodes, the main parts are a nerve ring or cephalic commissure, various ganglia and nerves associated with them. The nerve-ring and various associated ganglia may be compared with the 'brain' of higher groups of animals as they perform almost similar function. The lateral ganglia correspond to the cerebral ganglia and the circumentric ring represents their dorsal and ventral connections. The nerve-ring is in direct contact with the cephalic nerves, the dorsal ventral and lateral ganglion (Fig. 32). From the nerve-ring four cephalic nerves arise. Two of them are sub-dorsal and the other two are sub-ventral in position. These nerves proceed anteriorly and are

provided with ganglia at their origin just attached to the nerve-ring. These nerves run anteriorly all the way in direct contact with the external surface of the oesophagus. At the cephalic region they bifurcate and supply to the cephalic papillae. Five ganglia are present associated with the nerve-ring. They are called the dorsal cephalic ganglion, ventral cephalic ganglion, the post ventral ganglion and two lateral ganglia. Commissures connect the latter two with the nerve-ring on the one hand and the ventral cephalic ganglion on the other. Longitudinal nerves arise from each ganglion.

Four main nerves proceed posteriorly which originate from the nerve-ring and the associated ganglia. Namely, they are one dorsal nerve, one pair of lateral nerves and one ventral nerve. All are present in their respective chords. The lateral nerves arise at the base of the lateral cephalic ganglia. Each gives a branch to its corresponding cervical papilla and continues posteriorly. At the posterior region it bears a pair of large ganglia, the lumber ganglia (Fig. 30) in the anal region and finally terminate in the phasmids. Further, in the male, each of the caudal papillae receives a branch from the lateral nerves of its sides. The ventral nerve approach the tail and is the main body

nerve. The ventral nerve is paired in the beginning which later fuse to form a ganglion, called as the post-ventral or the retro-vesicular ganglion (Fig. 35). This proceed posteriorly as a single chord upto the anal region of the body. In the region of the vulva, this nerve supports the vulvar ganglion. Further posteriorly, the ventral nerve branches and the caudal muscles bend over to the ventral nerve for their innervation and bears two nerve ganglia a pre-anal ganglion at the level of the somato-intestinal muscles and an anal ganglion just anterior to the anus. A relatively large commissure passes on either side from the anal ganglion around the rectum to join with the rectal ganglion which lies on the dorsal side of the rectum a little anterior to the anal region. Differences have been noticed in respect of nervous system in the caudal region of the body in the two sexes. But they are of very minor importance and the arrangement almost remains the same in both the sexes. They can easily be homologized in most instances, if not entirely. An oesophago-sympathetic nervous system also occurs, in addition to the body nervous system.

The whole system has been divided into the following three parts in order to make the study easier.

1. Central nervous system
2. Peripheral nervous system
3. Caudal nervous system

Central nervous system

The central nervous system in the case of Heterakis gallinarum consists of a nerve-ring or cephalic commissure, various cephalic ganglia and the ganglionated part of the ventral nerve trunk.

Nerve-ring:

The nerve-ring, which is also commonly known as circum-oesophageal commissure, is present at the anterior part of the oesophagus at a distance of about 0.34 mm from the anterior part of the body. At this place the oesophagus is encircled by the nerve-ring. The dorsal side of the nerve-ring is slightly tilted anteriads and is somewhat inclined in position. It is composed of fibres and contains a few nerve cells, therefore, as such it must not be regarded as the main functional part of the nervous system (Fig. 32). It performs the function of an associated

structure where processes from the various ganglia of the central nervous system come in direct relationship with one another. Baker (1936) suggested that this structure largely consists of fibres but the present author is of the view that fibres of the nerve-ring are also supported and ensheathed by a substance of the nervous system known as glia. A net-work of tissues of subcuticular origin support the glia. The innervation processes from the somatic muscle in the cervical part of the body are also in direct contact with the nerve-ring, besides the regular nerve which will be discussed later. Associated with the nerve-ring are the ganglia of the cephalic papillary nerves, ganglia of the amphidial glands and the four cephalic ganglia, which are called a dorsal, ventral and a pair of lateral ganglia. There is one more ganglia in the ventral nerve situated a little behind the nerve-ring. This is known as post-ventral ganglia.

Cephalic ganglion:

Dorsal ganglion: According to Baker (1936) the dorsal cephalic ganglion is not very conspicuous and is the smallest of the anterior ganglia. It is situated on the inner side of the dorsal longitudinal band just behind the nerve-ring and embeded in the tissue of the dorsal chord

(Fig. 34). The characteristic 'radially striated' ganglion cell appearance is due to the fact that the glia fibrils actually enter the ganglion cell. The dorsal longitudinal nerve starts from this ganglion and continues posteriorly within the dorsal chord (Fig. 33).

Lateral ganglia: There are two lateral ganglia. They are by far the largest of the ganglia and are directly connected with the nerve-ring and the amphidial glands (Fig. 38). The author does not agree with Baker (1936) who observed that the lateral ganglion are closely associated with the inner regions of lateral chords, but instead these were observed enclosed by the lateral chord. Further, these extend inwards in this region to cover up these ganglia. These are situated laterally behind the nerve-ring, on either side. The ventral ganglion is joined by the lateral ganglia with the help of a minute commissures.

Ventral ganglion: The ventral cephalic ganglion lies at a little more posterior level to the nerve-ring than the dorsal cephalic ganglion on the ventral side (Fig. 38). From it passes two large masses of fibres to the nerve-ring (Fig. 37). It gives out commissures on either side. Further, they pass through the sub-cuticula to reach the two cephalic ganglia. The ventral longitudinal nerve arise

from the ventral cervical ganglion. This nerve posteriorly runs in the lateral chord.

Peripheral nervous system

The peripheral nervous system consists of amphidial nerves and somatic nerves.

Amphidial nerves:

Baker (1936) made no mention of this nerve in his studies. The amphidial nerves originate from the amphidial ganglion present in the lateral chords and anteriorly supply to the amphids (Fig. 36). There are present two pairs of amphidial nerves. The amphidial ganglia are situated in close association with the lateral ganglia posterior to the nerve-ring. Their connection with the nerve-ring is not direct though they innervate the amphid of its side. The cells of the amphidial nerves are situated in the amphidial ganglia, and the axones of the cells reach the nerve-ring by way of latero-ventral commissure.

Somatic nerves:

The nerves which run through various chords are collectively known as somatic nerves. The nerves involved are dorsal, a pair of lateral and a ventral nerve. They run in their respective chords.

Dorsal nerve: This is a median nerve and begins at the base of the dorsal ganglion from the posterior side of the nerve-ring. It runs through the length of the body embeded in the dorsal chord up to the pre-anal region. At pre-anal region it bifurcates, and both of its branches extend through the hypodermis to the lumbar ganglion forming the dorso-lateral commissures. It receives all the innervating processes from the somatic muscles lying on its either side in the dorsal half of the body. Hence it is to be regarded as motor nerves. No ganglia was observed throughout its entire length.

Lateral nerve: These are paired and one longitudinal nerve is lodged in each of the lateral chords. These arise at the base of the respective lateral cephalic ganglia and run towards the posterior end of the body just like dorsal and ventral nerves. Both of these nerves, their branches and various ganglia associated with them in the posterior

part of the body are involved in the formation of a complicated structure in the caudal end of the body, the so-called 'caudal nervous system'. They also function as motor nerves like those of the dorsal nerve which give innervation processes to the somatic muscles.

Ventral nerve: This nerve is situated opposite to the dorsal nerve in the ventral chord. It originates from the ventral aspect of the nerve-ring immediately below the ventral ganglia. In the region of the excretory bridge the ventral nerve bifurcates and the two branches go round the post-ventral ganglion and reunite towards its posterior region resulting in a single nerve which proceeds backwards passing to the right of the excretory pore. The ventral nerve also receives the innervations from the somatic muscle cells on either side, in the ventral half of the body. Thus it is also to be regarded as a motor nerve. It enlarges at its posterior region of the body, beyond the rectum, and is involved in certain ganglion formation.

Caudal nervous system

In the posterior or the caudal region of the body, (sometimes also termed as tail region) the ventral and lateral nerves with their various branches and ganglia

constitute a nerve-complex which form the 'caudal nervous system'. The arrangement of the ganglia and the nerve in this region remain the same in both the sexes. In case of male, fine nerve fibres arise from the lateral nerves to innervate the genital papillae and the genital sucker. These are the additional structure which are not present in the case of female. It is probably due to the increased functional activity of this region for the reproductive processes.

As the lateral and ventral nerves approach the caudal region or the tail, they become much thicker. It is present in the region of the somato-intestinal muscles. Proceeding posteriorly, a little anterior to the anus, the ventral nerve terminates in a ganglion known as the anal ganglion. It lies a little to the left of the anus and not exactly ventral in position. From either side of the anal ganglion, the ano-rectal commissure arise which is in the form of relatively fine bundles of fibres. It passes round the rectum and joins the rectal ganglion which is present on the dorsal side of the rectum.

Structure of the ganglion cells:

The structure and size of various ganglion cells vary in different regions. Some are unipolar and some are

bipolar, median sized or large cells. In 1908 Goldschmidt made an extensive study of ganglion cells in Ascaris. He observed that the ganglion cell is typically divided in three zones. This condition also appears to be true in the present worm. The outer zone which borders the cells is rather a coarse and alveolar structure. The middle layer is in direct contact with the nerve processes and also small alveoli. Finally the innermost layer which lies immediately round the nucleus is dense in nature. In different ganglia these cells undergo modification. A large cell which is peculiar in having a fairly good quantity of glia substance surrounding the actual cell is present in the dorsal ganglion (Fig. 41). The glia fibrils enter the ganglion cells in such a manner as to give it the appearance of being made up of radiating structures.

Innervation of muscles:

Schneider (1860), after extensive studies expressed his opinion that in nematodes the muscle 'seek the nerves' while in other groups of animal nerves go to muscles. Apathy (1894) believed the innervation processes, as 'interistitial muscle'. He also observed in it centrally located neurofibrillar structure. This made him to conclude that median somatic nerves give rise to neurofibrillar net

work. These proceed through the innervation processes and supply to the muscle cells. At this point it turns back the same way and returns to the nerve of its origin. Goldschmidt (1910) was of the view that neurofibril is in direct contact with the central field of each innervation process.

A proper and final word regarding the contact of neurofibrils with the fibrillar net-work in the sarcoplasm is still awaited as the workers differ in this respect. But there is no doubt that the innervation processes are in direct contact with the sarcoplasmic portions of the muscle cells and there is no difference of opinion regarding the presence of fibrillar net-work in the sarcoplasm.

Receptor organs

1. Amphids
2. Caudal papillae
3. Phasmids

Amphids:

The amphids or the so-called lateral organs are a pair of special sense organs. They lie laterally and believed to function as chemoreceptors (Fig. 42). When

the structure and function of the amphids were not established, the earlier workers erroneously mistook them for papillae. The amphidial glands open as circular openings. The amphidial ganglia give rise to amphidial nerves and extend anteriorly in the amphids where these end in the form of a cluster of nerve fibres.

The dilated portion of the gland is known as the amphidial pouch and is present posterior to the nerve-ring in the dorsal part of each lateral chord. The nucleus of the amphidial gland is located at this region. The amphidial gland open to the exterior through their ducts to the amphidial pore.

Caudal papillae:

In the present worm there are in all 11 pairs of caudal papillae, present in the case of males only. The papillae is like a pointed knob which projects outwards. Each papilla is provided with a minute pore which ultimately leads into a canal having a supporting cell. The lateral nerves innervate these papillae and run in the sub-cuticula very close to the inner side of the canal terminating in a knob-like structure. Probably, these control the discharge of the sperms. The lumbar ganglion innervates the sucker and the caudal alae.

Phasmids:

Like those of the amphids, the phasmids are also placed on the lateral sides but in the region of the tail. They resemble the amphids in structure and their external manifestation is like pore. Each of the phasmid is also provided with a unicellular gland known as 'phasmidial gland'. A short duct passes anteriorly through the cuticle from each pore, running anteriorly to open into the gland of its side. The lateral nerves terminate in the phasmids and thus innervate them (Fig. 40).

Explanation of figures

Fig. 32. T.S. passing through the nerve ring and lateral ganglion.

Fig. 33. Reconstruction of the dorsal ganglion.

Fig. 34. T.S. passing through the dorsal ganglion.

Fig. 35. T.S. passing through the post-ventral ganglion.

Fig. 36. Reconstruction of the lateral ganglion.

Fig. 37. Reconstruction of the ventral ganglion.

Fig. 38. T.S. passing through the ventral ganglion.

Fig. 39. T.S. passing through the anal, rectal and lumbar ganglia.

Fig. 40. T.S. passing through the phasmid and phasmidial glands.

Fig. 41. T.S. through the ganglion cells.

Fig. 42. T.S. passing through the amphidial pore.

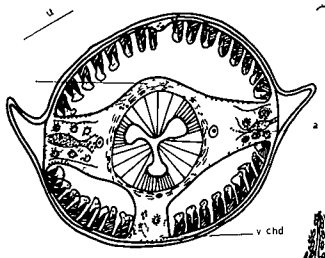
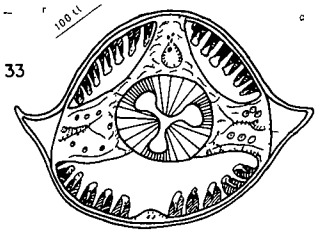


FIG 32

FIG 33



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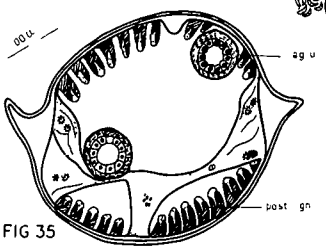

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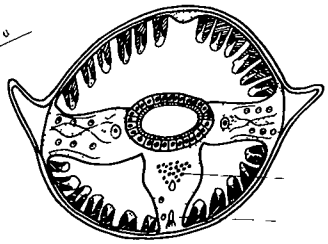


FIG 38

FIG 37

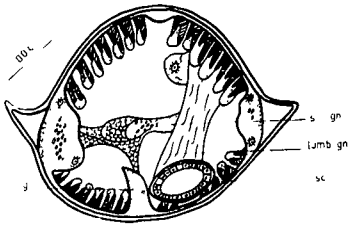


FIG 39

FIG 40

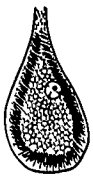


FIG 41

FIG 42

REPRODUCTIVE SYSTEM

Female reproductive system

Female reproductive system in Heterakis gallinarum, comprised of vulva, ovijector, vagina, two uteri, two oviducts and two ovaries. The vulva is situated at about the middle of the body at a distance of about 5.90 mm. It is more or less a rounded aperture, situated on slight elevation (Fig. 43). It leads into an anteriorly directed ovijector. It is the specialized region of the gonoduct with communicates with the U-shaped vagina. The vagina is directed anteriorly for a considerable distance and turns again till it meets the two uteri (Fig. 46). The uteri are thin-walled, and occupy a large part of the body space both in the anterior and posterior region. At the level of the vulva the anterior uterus joins posterior ovary through the posterior oviduct. Similarly the posterior uterus joins the anterior ovary via anterior oviduct. The anterior oviduct is shorter than its posterior counterpart. The anterior ovary during its course towards the anterior region shows its characteristic looping and coiling arrangement. Similarly the posterior ovary is also characterized by its looping arrangement but its diameter decreases considerably during its course. The

available space of the body cavity is almost occupied by various parts of the genital system.

Ovary

Both anterior and posterior ovaries are characterized by their looping arrangement. According to Baker (1936) the ovaries are composed of thin and somewhat flattened cells but the author feels that each ovary is composed of an epithelial layer and a germinal chord and is characteristically looped. The tip of the ovary is covered by a small cell known as the 'cap cell' (Fig. 52). Baker (1936) used the term 'ovary tip' to include all of the more or less straightened and narrower distal terminations of the ovaries. He did not give any account of the cap cell. Nature of the cap cell is open to question. Many earlier workers did not believe in its presence as a separate existence as they presumed it to be an ordinary epithelial cell of the ovarial terminus. Chitwood (1929) also described this cell as a part of the ovarial epithelium. Musso (1930), on the other hand gave a completely different view. He considered cap cell as indifferentiated germinal stem cell. He observed that both the epithelial and the germinal cells equally arise from this germinal stem cell. Therefore, it is regarded

as a purely epithelial structure which has nothing to do with the germ chord.

In Telogonic forms such as Heterakis gallinarum, each ovary is divisible and consist of two distinct zones.

The germinal zone is located at the terminal end of the ovary which is also known as "Keimzone". This zone is followed by a growth zone or "Wachtenzone".

Germinal zone: This zone starts from the ovary tip and runs in a posterior direction for a short distance till it meets the growth zone (Fig. 47). The ovary remains characteristically looped. About half the length of the ovary is occupied by the germinal zone. The germinal zone is an area of rapid division of relatively small germ cells. The author confirms the observations of Baker (1936) who reported that the cells of the extreme tip of the ovary are spherical in shape but tend to become flattened due to the pressure caused by the increasing number of the cells. The cell boundaries are visible to a considerable extent though the cell border are difficult to distinguish at the germinal end of the zones, but well distinct cell walls are present. Hence the region is definitely cellular and not syncytial though in some other instances, it has earlier been reported. It is covered by very thin epithelial layer.

Growth zone: The germinal zone continues into the growth zone towards its proximal end and then runs anteriorly. This is the region where the mitotic activity takes place and the gametogonia grow and increase in size and differentiate (Fig. 53). The growing cells of this region are covered by the peritonial layer and they occupy the entire cavity. The oogonia are closely packed and they are conical in shape. Being a telogonic nematode, Heterakis gallinarum, has a 'rachis' which is a characteristic feature of this group of nematodes. The fused ends of the oogonia appear like the spoke of a wheel, fastened by their fused ends or 'rachis'. The wall of each of these cells is in the form of a thin membrane having a granular content. There is a large nucleus with a prominent deeply staining nucleolus in each cell. The nucleolus is spherical in shape. The epithelial layer of this zone is much thicker than that of the germinal zone of the ovary.

Oviduct

The proximal end of each ovary opens into the oviduct (Fig. 48). The anterior ovary opens into the anterior oviduct and joins the posterior uterus, while the posterior ovary opens into the posterior oviduct and joins the anterior uterus. The oviducts are in the form of short and narrow

tubes. Vogel (1925) was of the opinion that oviducts should be regarded as an egg former and possibly a shell gland, but later workers did not confirm this idea. The muscular and epithelial layers of the oviduct increase in thickness and enlarge at its proximal end. The author do not agree with Baker (1936) that the oviducts are capable of slight contraction but believes that the projections of the epithelial cells help in the forward movement of the eggs. Rauther (1918) described a muscular sphincter at the ovary-oviduct junction in Macracis but no such structure was observed in Heterakis gallinarum.

Uterus

The uterus is the most complicated part of the entire female reproductive system as two uteri (anterior & posterior) occupy most of the available space in the body cavity on either side of the vulva. The anterior oviduct opens into the posterior uterus and the posterior oviduct opens into the anterior uterus. The uterus is in the form of a wide sac which contains a large number of developing eggs (Fig. 45). According to Baker (1936) the uteri are very thin walled but the present author believed it to be comprised of a single layer of epithelial cells with distinct

and fairly large nuclei. There is a considerable difference between the wall of the uterus and the genital tube. It consist of two layers, the external membranous layer of contractile cells and an inner layer of low epithelial cells. The cells of inner epithelial layer are spirally arranged. The eggs are carried by the peristaltic contraction waves passing along the inner spirally arranged epithelial cells. There is no muscle supply throughout the length of the uterus, although Cobb (1923) as well as other authors have described it in certain nematodes. The present author confirms the presence of a fluid within the uterus which is precipitated by the fixative. This could be observed in sectioned materials in the form of a gelatinous mass. It helps in the movement of the eggs. According to Magath (1919), this fluid is supposed to provide nourishment for the growing eggs. Heterakis gallinarum is oviperous and hence the uteri are full of eggs. The uterus functions as a passage and also as a storage place. Finally the uteri opens into the vagina.

Vagina

The uteri communicate with the ovijector through a muscular tube known as vagina (Fig. 50). It is divisible into two portions the vagina uterina and the vagina vera.

Vagina uterina: It is in the form of an elongated tube which extends posteriorly and is again reflexed towards the anterior before connecting with the amphidelphic uteri. The present author confirms the presence of a semi-fluid as earlier reported by Baker (1936). Eggs and spermatozoa were also observed in this region but their number is far smaller than those present in two uteri. The wall of the vagina uterina is comprised of two distinct layers, an outer muscular layer consisting of circular muscle fibres and an inner epithelial layer which is continuous with that of the uterus and is of the same general type but forms a layer of several cells in thickness. This causes the vagina to have a distinctly laminated appearance.

Vagina vera: The terminal portion of the vagina is known as the vagina vera. It is in the form of a short tube. Regardless of degree of development, the vagina is lined with a distinct, well developed cuticular layer continous with the external cuticle of the body.

Ovijector

The terminal muscular portion of the genital tube opens into the ovijector through vagina vera and is known as ovijector (Fig. 51). This acts as the ejector of the eggs.

In addition to ordinary muscles there is large "sphincter muscle" near the vulva opening in the form of circular muscles. They probably also function during the act of copulation. It is not clear whether or not they control any movement of the vulvar lips. The ovijector is lined throughout except for a short distance at either ends, with two layers of cellular material. The shape of the cells is unique being more or less rounded in cross-section and spindle shaped longitudinally. Due to the appearance of more cells at the inner end of the ovijector the rows become irregular and not well marked.

The ovijector extends anteriorly towards the vulva and suddenly makes a very sharp turn within the ventral part of the body-cavity, loses its musculature and passes posteriorly to open near the left of the median line.

The vestibule and the vulva:

At the junction of the ovijector with the vulva, a very interesting cuticular structure is present, which from its position and structure is considered as valve, opening by its own elasticity and closed by the action of the sphincter. There is further possibility that they serve in some way during the act of copulation, e.g. in holding the

spiculum. It is not certain whether this valve is of general occurrence among parasitic nematodes or exists in the present worm only.

The vestibule, like the ovijector is also ectodermal in origin and is in the form of a short tube of very little diameter and will allow the passage of only one egg at a time.

The vulva is in the form of a transverse slit situated on the ventral side of the body wall at a distance of 5.90 mm from the anterior end. The body wall shows distinct elevations to form lip-like regions on either side of the vulva. The opening is in the form of a bulging which distinctly protrudes outside the body to the left of the ventral median line.

Explanation of figures

Fig. 43. T.S. passing through vulva, vulvar muscles and vulvar ganglion.

Fig. 44. T.S. passing through the vagina vera and sphincter muscles.

Fig. 45. T.S. passing through the middle region of the body showing ovary, oviduct and seminal reservoir and uterus.

Fig. 46. Diagrammatic reconstruction of the female reproductive system.

Fig. 47. T.S. passing through the germinal zone of the ovary.

Fig. 48. T.S. passing through the oviduct.

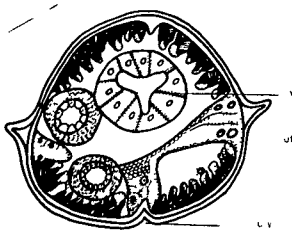
Fig. 49. T.S. passing through the seminal reservoir.

Fig. 50. T.S. passing through vagina-uterina.

Fig. 51. L.S. of vagina vera.

Fig. 52. T.S. passing through the germinal zone of the ovary showing the cap cell.

Fig. 53. T.S. passing through the growth zone of the ovary.



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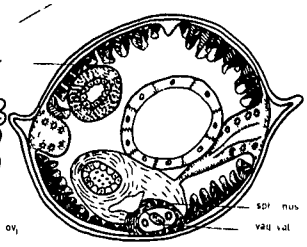

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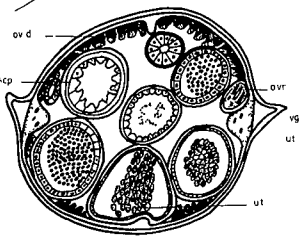


FIG 45

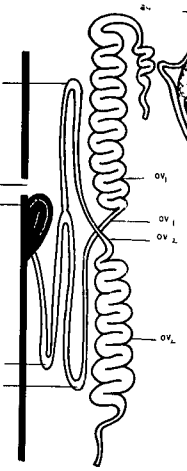


FIG 46

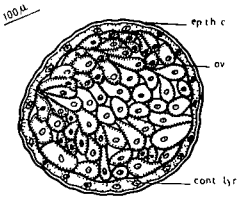


FIG 47

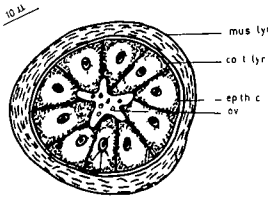


FIG 48

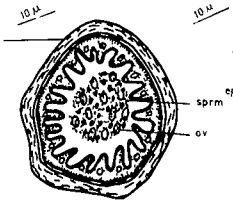


FIG 49

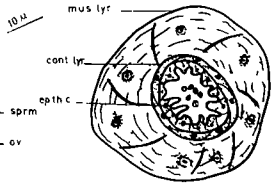


FIG 50

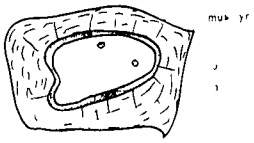


FIG 51

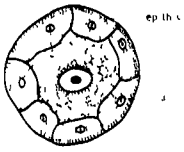


FIG 52

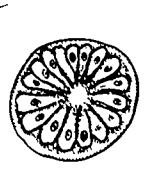


FIG 53

Male reproductive system

The male reproductive system is more or less in the form of a tube. It begins with a single testis, hence the worm is monorchic, which is irregular and is present in the anterior region of the body. It continues after a sharp constriction into a small and broad seminal reservoir. The latter narrows towards its distal end and is known as the vas deferens. Vas deferens followes into the ejaculatory duct. An ejaculatory valve is also present inside the duct. The region which lies anteriorly to the ejaculatory valve is termed as ejaculatory vesicle. Two accessory glands are situated (Fig. 57) on either side of the proximal end of the ejaculatory duct, at the point where it enters the cloaca. These are in the form of small glands. The caudal alae, spicule, and sucker are present in the posterior part of the body which are considered to be helpful in the act of copulation.

Testis

The single testis (Fig. 57) of the male is present in the anterior region of the body and proceeds posteriorly parallel to the oesophagus upto the anterior enlargement of the intestine. It is more or less in the form of a tube which

shows several bends and loops during its course. The cells of the testis are very much flattened and have a rather heavy outer basal membrane. Like the ovary it is also divisible into two regions, an anterior germinal zone followed by a growth zone.

Germinal zone: The very anterior end of the testis, which ends blindly is known as the germinal zone (Fig. 68). It is in the form of a tube composed of single layer of epithelial cells. Proximally the number of cells increase in this zone till there are many cells in one cross-section. Unlike, the germ cells of the ovary which divide radially resulting in the formation of only one layer of cells encircling the ovarian tube, the germ cells of the male divide in more than one plane resulting in the formation of more than one layer within the testicular tube. The primary germ cells are spherical in outline with a distinct nucleus in the centre. The germ cells are compactly packed in it. There is no line of demarcation between the various layers as all the available space is occupied by these cells. Proximally the tube is continuous with the growth zone of the testis.

Growth zone: As has already been described, the growth zone is continuous with the preceeding germinal zone of the testis and there is no distinct structure to separate or divide this

part from the preceding germinal zone (Fig. 69). As a matter of fact the latter passes rather inconspicously into it. The covering epithelial layer of this region is also continuous with that of the germinal zone. However, this layer is comparatively slightly thicker here. All the available space is occupied by large mature cells ready to undergo division. The cells contain a big spherical nucleus in the centre, with a distinct nucleolus. The cells become elongated and develop into spindle-shaped spermatozoa after the division is over. The spermatogonia are attached to a rachis. The structure is apparently a process from the cap-cell or terminal epithelial cells of the testis similar to that of the ovary. This distal region of the testis is covered with an epithelium continuous with that of the seminal reservoir. The character of the epithelial layer changes as we proceed from the proximal to the distal end of the testis, consisting of flat cells in the beginning and changing into a layer of high columner cells near its end.

Seminal reservoir

The seminal reservior is a bit modified sac-like enlarged portion of the system (Fig. 56). Here the testis discharge its contents. Sometimes, it has also been referred

to, as an organ for the storage of the sperms, before copulation has taken place. The cells here are free from rachis. The walls of this region are in direct continuation with those of the testis and as a matter of fact, the seminal vesicle is itself a dilated portion of the male gonoduct serving as a temporary store-house for maturing sperms passed out by the testis. As normally believed, the function of the seminal reservoir is not to store the spermatozoa but to pass them on to vas deferens though it certainly acts as a temporary storehouse for maturing sperms. The size of this region is determined chiefly by the amount of germinal products present in it. The wall of the anterior region is provided with simple squamous cells while cells of the posterior part are vacuolated. The extended processes of the epithelial cells later become thickened into the lumen of the seminal reservoir. Probably these projections help in pushing and carrying the sperms downwards.

The nature of spermatozoa has been a matter of controversy as opinions differ. These products are considered by most of the workers as immature and attain maturity unless these remain for sometime in the uterus of the female. Looss (1905), however, speaks of mature spermatozoa in this region of the system in Ancylostoma duadenale. The present author agrees with the suggestion of Looss, as fully developed

and mature spermatozoa were observed in Heterakis gallinarum while still within the body of male. These were found to be non-flagellate in form and almost sub-oval in shape. These apparently have, their blunt heads pointing anteriorly and a pointed posterior end. Ectoplasm is thin and cytoplasm is vacuolated having a distinct centrally placed nucleus (Fig. 58 & 59).

Vas deferens

The seminal reservoir continues towards its distal end forming a narrow tube known as the vas deferens (Fig. 54, 65, 66 & 67). Two unequal ejacuolatory glands are also present at this region. It is considered to be an important part of the sperm duct.

The author agrees with Baker (1936) that vas deferens consist of a single layer of cells. In majority of nematodes the vas deferens has a rather high simple cuboidal to columnar epithelium. The cells are glandular due to the presence of secretory mass in the epithelial cells lining the duct (Fig. 65). In the case of Heterakis gallinarum, the epithelium too, seems to indicate a glandular function. Hair-like processes were not observed in the present case, though Cobb (1923) and Rauther (1909) had earlier reported such a structure in some nematodes.

Ejaculatory duct

The vas deferens is followed by a duct which is known as the ejaculatory duct and is the terminal portion of the male gonoduct. At its anterior end, immediately following the vas deferens, it is quite narrow but attains maximum width after a short distance. It then gradually tapers down at its posterior extremity, and joins the ejaculatory vesicle. The wall of the ejaculatory duct is lined by a layer of thick and high epithelial cells (Fig. 55). The junction of the ejaculatory duct with the ejaculatory vesicle is guarded by a well developed valve.

Ejaculatory vesicle

The posterior region of the ejaculatory duct is termed as the ejaculatory vesicle. It is composed of two layers, the outer circular musculature containing a few nuclei, and project a little on either side of the vesicle. The muscle layer is thin and probably acts as a muscle to produce peristaltic motion, and functions in the removal of the spermatic fluid from the male generative organs. The inner layer is composed of tall columnar epithelial cells. It is suggested by some authors that they secrete some gelatinous substance at the time when copulation takes place which

accompanies the sperm into the uterus of the female. It is not known whether this fluid is nutritive or merely a mechanical carrier for the sperms. Some fluid medium was necessary for conveying the male cells into the female and it seems most logical to believe that this organ may secrete such a fluid. The author agrees with Magath (1919) who rejected the suggestion of Looss (1905) that this organ secretes a cement which helps to stick the male with the female during copulation.

The accessory glands of the ejaculatory duct: Perhaps there is no point as controversial in the entire nemic morphology than the significance and function of the so-called accessary glands of the ejaculatory duct. They are probably of common occurrence among parasitic nematodes but have been overlooked in some cases by many workers. The author agrees with Baker (1936) who assumed, on purely theoretical grounds, that the glands secrete a fluid which activates the sperm cells when these leave the gonoduct of the male. These discharge into the ejaculatory duct at a point ventral, and slightly anterior, to the two latero-ventral rectal glands of the male.

In view of the observations it is very difficult to ascribe any definite function to these glands. Their close association with the sphincter muscles would, on the other hand suggest that they might be a component part of the sphincter itself.

Spicules

In case of Heterakis gallinarum, there are two unequal and dissimilar spicules. Each spicule is covered by a sclerotized cuticle containing a central protoplasmic core. It was observed that its cuticular covering is continuous with the cuticular lining of the spicular pouch. Usually the left spicule is larger than the right but in Heterakis gallinarum the converse is the case. Here it was observed that the right spicule, is the larger of the two, and generally tapers into a fine sharp point (Fig. 70) at its end. The left spicule (Figs. 62 & 71) is comparatively much shorter about 0.78 mm in length, heavier and often terminates in a massive hook. The right spicule, the larger of the two is 1.23 mm long. It is almost alate in shape and narrows rather suddenly, near its distal end to a sharp point. A longitudinal groove is present on its sides almost throughout its entire length. Baker (1936) observed that the right spicule extends for a considerable distance forward on the right side of the body cavity. In cross-section this spicule shows the same appearance almost throughout its whole length.

Baker (1936) pointed out that the spicules are essentially cuticular structures, but all the layers of the

body-wall are not represented here. It appears that spicules consist of two distinct cuticular layers. The inner layer is filled with a granular mass having dark staining property (Fig. 67, 73). This granular mass was termed as "granular pulp" by Magath (1919). Both the spicules are separately enclosed in a muscular 'spicular sheath'. It consists of retractor muscles and the exertor muscles. The muscles of this sheath are responsible for the movement of the spicules during copulation.

The retractor muscles of the spicule: These muscles are connected with the spicule from the anterior side but the exertor muscles take their position slightly towards the sides of these regions. It was observed that the general arrangement of the muscles remain the same with both the spicules.

Starting from the head of the spicule (Fig. 63) the retractors run anteriorly. It has a centrally placed granular core consisting of a single hollow muscle but this structure is present for a very short distance. Immediately after, the single tube-like muscles show a tendency to divide throughout its entire length, forming two separate tube-like structures but structurally they are similar (Fig. 60, 61).

The retractor muscles associated with the left spiculum (Fig. 61) are more or less similar to those obtained in the case of right spiculum having almost the similar arrangements. These muscles run towards the anterior end in a single strand of material which is finally inserted on the ventral side of left lateral chord at a place where the central valve makes its appearance in the ejaculatory duct. Both the retractor muscles of the right and left spiculum are inserted in the lateral chords and they have never been observed inserted above the lateral chords.

The exertor muscles of the spicule: The exertor muscles almost cover the whole of the spicule from all its sides. The arrangement remains the same for both the spicules. The muscles which are present around the spicules are known as the "spicular sheath". The sarcoplasmic elements were also observed but they are present outside the fibrillar portion. Looss (1905) and Magath (1919) also observed similar type of muscular elements.

There is no sign of attachment of any kind along their course except at the ends. They remain free, enclosing the spicule and lie on either side of the body cavity.

Unlike those of the retractor muscles, the exertors of the spicule are inserted on the inner ventral wall of the body after a short distance posterior to the anal opening.

The spicular canal: The spicular canal arises from the cloaca from its dorsal side and runs for a short distance from the anal opening and is lined with cuticle (Fig. 64). At the time of copulation, the spicules emerge from this canal. In the beginning the canal was observed to be single but after a short distance it becomes paired. In the case of Heterakis gallinarum, the spicular canal is lined with fine cuticula and the transverse markings which are present in the body wall are also very prominent.

The spicular canal runs forward carrying the two spicules, the short spiculum is placed dorsally to the long spiculum. Anteriorly, the spicular canal also divide at a place where the two spicules are separated to occupy their respective place on the two sides of the body cavity.

Explanation of figures

Fig. 54. T.S. passing through the vas deferens.

Fig. 55. T.S. passing through the ejaculatory duct.

Fig. 56. T.S. passing through the seminal reservoir.

Fig. 57. Diagrammatic reconstruction of the male reproductive system.

Fig. 58. L.S. spermatozoa.

Fig. 59. T.S. spermatozoa.

Fig. 60-63 Details of the heads and muscular attachment of the spicula.

Fig. 60 & 62 Short spiculum.

Fig. 61 & 63 Long spiculum

Fig. 64 T.S. passing through the accessory glands and spicules.

Fig. 66 T.S. passing through the vas deferens and spicules.

Fig. 67. T.S. passing through the vas deferens and rectal gland.

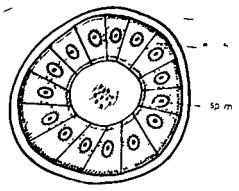


FIG 54

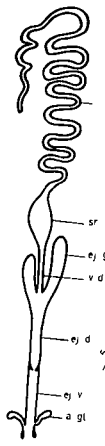


FIG 57

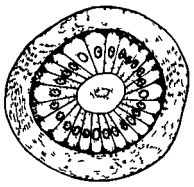


FIG 55

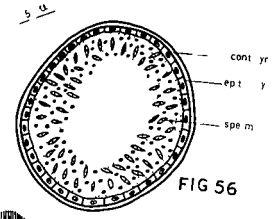


FIG 56

FIG 58

FIG 59

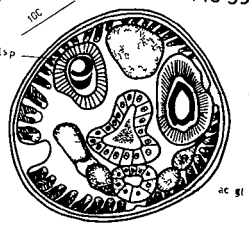


FIG 64

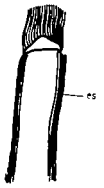


FIG 60

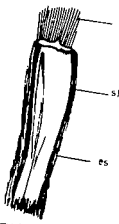


FIG 61

FIG 62

FIG 63

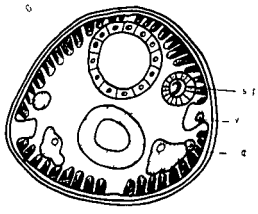


FIG 65

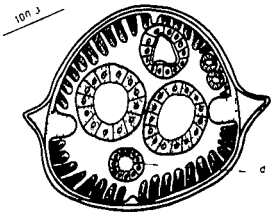


FIG 66

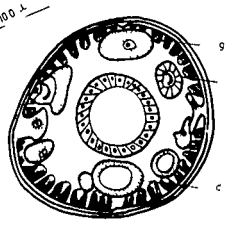


FIG 67

Explanation of figures

Fig. 68. T.S. passing through the germinal zone of testis.

Fig. 69. T.S. passing through the growth zone of the testis.

Fig. 70. Distal tip of right (long) spiculum.

Fig. 71. Distal tip of right (short) spiculum.

Fig. 72. T.S. passing through the spicules, cloaca and sucker muscles.

Fig. 73. T.S. passing through the cloaca and spicules.

Fig. 74-81. Developmental stages of *Heterakis gallinarum in vitro*.

Fig. 75. Morula stage of embryo.

Fig. 80 & 81. Vermiform embryo inside the egg shell.

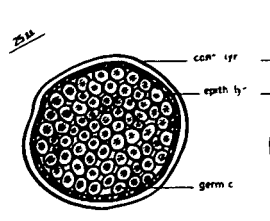


FIG. 68

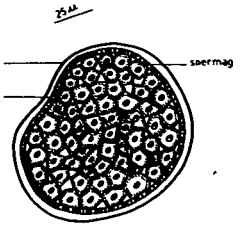


FIG. 69

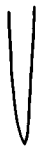


FIG 70

FIG. 71

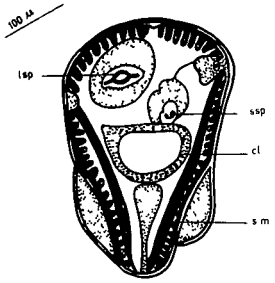


FIG. 72

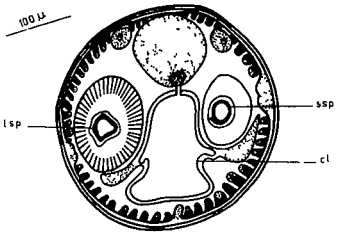


FIG 73

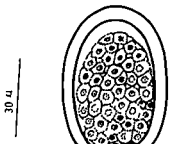


FIG 74

FIG. 75

FIG. 76

FIG. 77

FIG 78

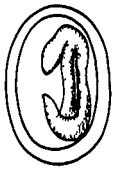


FIG. 79

FIG. 80

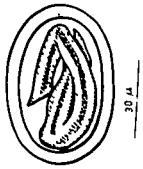


FIG 81

P A R T - B

B I O L O G Y

LIFE CYCLE

Life cycle studies of Heterakis gallinarum, has been, in the past, a subject of extensive investigation due to conflicting views. Different opinions have been expressed on its embryonic and post-embryonic development. Generally the life cycle was believed to be direct, but later findings revealed that it may be indirect also involving intermediate hosts like gross hoppers and earthworms etc.

Development outside the host is greatly influenced by the environment and one of the outstanding functions that determine the possibility and rate of development to the infective stage is temperature. Heavy growth of bacteria and fungi also seem to interfere with the development, retarding it and causing many of the eggs to disintigrate. Further, there are certain chemicals which have profound effect on the process of embryonation and enhance favourable development of the eggs to the infective stage.

The studies reported herein were taken up on the following lines.

1. Embryonic development *in vitro*.
2. Migratory course of the juveniles *in vivo*.
3. Tissue response.
4. Indirect life cycle.
5. Incidence of infestation and seasonal abundance.

Embryonic development in vitro.

Heterakis gallinarum ova undergoes no development in the caeca of the host and pass out in the single celled stage. Different culture media were used for the incubation of ova and observations on the development at variable temperatures from 21 to 40°C were made. The results obtained are as follows.

First division of the single celled stage in normal saline maintained at 21°C took place after 24 hours. During the first stage of the cleavage egg appeared exceedingly granular and the nuclei of the two resulting blastomeres were peripherally located. In most of the cases the resulting blastomeres divided simultaneously. It was quickly followed by further divisions forming a morula (Fig. 75) in 5 days. The activity continued for seven days

and in the meantime a depression formed between the two types of blastomeres. The ova grew terminally transforming it into a vermiform embryo (Figs. 74 to 81). Although the whole process was completed in 15 days but it got retarded sometime due to the growth of the fungus. At temperatures from 23°C to 27°C no significant change in the development was apparent, but at 30°C the rate hastened quite significantly and first division took place within 23 hours and the whole process was completed in 13 days with an average yield of 21% of viable eggs. Above this range of temperature development proceeded rapidly with first division taking place within 20 hours; morula stage reached in 3 days and complete development obtained in 11 days. The development completely stopped at 40°C.

In media with 1% formalin maintained at 21°C first division occurred in 24 hours, morula stage was produced in 4 days and development completed in 17 days. At 30°C first division of the single celled stage took place in 3 days. The activity continued for the next five days. Then after 3 days of rapid development, the embryo grew terminally with a vermiform embryo. About 37% of eggs embryonated. The whole sequence was completed in 13 days. The development proceeds rapidly at higher temperatures,

but only few eggs embryonated and rest got perished.

In distilled water at 21°C the first division occurred after 24 hours and the whole sequence was completed in 16 days. It was observed that a speedy growth of fungus took place in this medium and development got retarded. Development at 30°C enhanced and was completed in 14 days, but due to the growth of fungus yield of the viable eggs was very low. The development continued to retard at the higher temperatures.

Development in Nitric acid was slow at 21°C, but at 30°C increased rapidly and completed within 16 days. About 27% of eggs were obtained which was a bit higher in comparison to other media. Development in Potassium dichromate was very slow at 21°C and the whole sequence was completed in 19 days. Development at 30°C improved a little and took 17 days. The yield was also low as 19% only. No development occurred in Hydrochloric acid, Sulphuric acid, acetic acid, Phenol, Corrossine sublimate and 70% alcohol. Some of the eggs were promptly destroyed, few began to develop, which later started to show degenerative change and all failed to attain complete development.

Percentage of the egg which embryonated in the media normal saline, formalin and nitric acid appeared to be

quite satisfactory. Uribe (1922) was able to maintain Heterakis ova in 1.5% nitric acid for 4 months in viable form which correspond to the present observations. But his approach to obtain embryonation in 10 - 15% formalin was not successful, the ova soon showed degenerative changes. Probably this was because he used fairly high percentage of formalin. The present study revealed that 1% formalin in distilled water proved to be the best culture media because the yield of embryonated ova was comparatively the highest of all the media used. Ackert (1931) also reported successful results with the use of formalin solution. Lund (1958) used physiological saline, 1.5% nitric acid and 1% formalin as incubation media for Heterakis ova. The media permitted 27.3%, 25.2% and 31.0% embryonation of the eggs respectively. The present observations come very close to these findings.

Distilled water and potassium dichromate which provided promising embryonation initially, but due to the inherent disadvantage, the yield of viable ova was found to be very low. The former due to the growth of mold which got the embryonation retarded, and the latter because of the tough shell, the embryo failed to hatch. Uribe (1922) also used 2% potassium dichromate solution for incubation of ova but

failed to get any viable eggs, but addition of few drops of formalin brought quite encouraging results. Vigdor (1918) reported that potassium dichromate offered a suitable medium for ascarid egg development and other coccids. it is strange that Heterakis ova incubated in this solution failed to produce infection; whereas spontaneous hatching of oocyst occurred in the same medium. Eggs of Heterakis has been found to survive for long period of time which indicated that the solution had little direct toxicity on the developmental stages. Lund et al. (1958) also reported satisfactory embryonation of Heterakis ova in 2% dichromate solution, but recovery of adult worms from chickens infected with these eggs was very low. The authors believed that the embryos failed to hatch because of the tough shell.

Optimum temperature for incubation appeared to be 30°C. Lower temperature limit between 21°C to 40°C presented slow development and eventually the embryonation period was prolonged. Development hastened by raising the temperature to 33°C and infective stages could be obtained within 10-12 days. Further rise in the temperature was determined and all development eventually stopped at 40°C. Uribe (1922) made comparable observations on the temperature requirements for the experimental incubation of the ova of Heterakis papillosa.

He observed that ova at room temperature embryonated within 9-12 days. He also believed that the development at room temperature (19-21°C) was as rapid as at 42°C. Clapham (1933) reported that the wide range of temperature, 20-30°C was able to support development and 26°C proved to be the optimum temperature.

Migratory course of juveniles in vivo:

Young chickens fed approximately 200 embryonated ova (17-day old) for the study of developmental cycle within the host body. These were sacrificed at 4 hours interval and organs were examined for the developing stages. Observation made from the initial infection till the recovery of adult worm are presented in (Table 1). Hatching of the ova took place in the proximal portion of the intestine in 2-3 hours. Within 4 hours juveniles were located in the pharynx and crop regions. Second molt occurred in the intestine within 48 hours. Juveniles, in the 3rd stage reached caeca within 168 hours. These disappeared suddenly from the caeca and after a lapse of 50-60 hours, appeared again in the lumen of the caeca with the completion of the third molt within 224 hours of the initial infection. During the next 23-30 hours these passed through the 4th

Table 1. Experimental infection of chickens with embryonated eggs of Heterakis gallinarum and recovery of the juveniles/adults.

No. of bird	Hours between initial feeding and autopsy	Site of recovery	No. of juveniles/ adults
1.	4 hours	Pharynx	1 Juvenile
2.	8 "	-	-
3.	12 "	Crop	2 "
4.	16 "	Crop	3 "
5.	20 "	Duadenum	2 "
6.	24 "	-	-
7.	28 "	-	-
8.	32 "	-	-
9.	36 "	Near caeca	3 "
10.	40 "	Near caeca	5 "
11.	44 "	-	-
12.	48 "	Intestine	2 "
13.	52 "	-	-
14.	56 "	Near caeca	2 "
15.	60 "	-	-
16.	64 "	Intestine	1 "
17.	68 "	Intestine	1 "
18.	72 "	Caeca	2 "
19.	76 "	-	-

(Continued)

Table 1. (Continued)

No. of bird	Hours between initial feeding and autopsy	Site of recovery	No. of juveniles/ adults
20.	80 hours	-	-
21.	84 "	Caeca	1 Juvenile
22.	88 "	-	-
23.	92 "	Intestine	1 "
24.	96 "	Caeca	3 "
25.	100 "	Caeca	3 "
26.	104 "	Caeca	2 "
27.	108 "	Caeca	6 "
28.	112 "	Intestine	1 "
29.	116 "	Caeca	5 "
30.	120 "	Caeca	2 "
31.	122 "	Intestine	1 "
32.	126 "	Caeca	3 "
33.	130 "	Caeca	3 "
34.	134 "	-	-
35.	138 "	Caeca	11 "
36	144 "	Caeca	1 "
37.	148 "	Intestine	12 "
38.	152 "	Caeca	12 "
39.	156 "	Caeca	15 "

(Continued)

Table 1. (Continued)

No. of bird	Hours between initial feeding and autopsy	Site of recovery	No. of juveniles/ adults
40.	160 hours	Caeca	13 Juveniles
41.	164 "	Caeca	15 "
42.	168 "	Caeca	13 "
43.	172 "	-	-
44.	176 "	-	-
45.	180 "	-	-
46.	184 "	-	-
47.	188 "	-	-
48.	190 "	-	-
49.	196 "	Caeca	2 "
50.	200 "	-	-
51.	204 "	-	-
52.	208 "	-	-
53.	210 "	-	-
54.	216 "	-	-
55.	220 "	-	-
56.	224 "	Caeca	3 "
57.	228 "	Caeca	21 Adults
58.	232 "	Caeca	11 "
59.	236 "	Intestine	11 "

(Continued)

Table 1. (Continued)

No. of bird	Hours between initial feeding and autopsy	Site of recovery	No. of juveniles/ adults
60.	240 hour	Caeca	13 Adults
61.	244 "	Intestine	12 "
62.	248 "	Caeca	15 "
63.	252 "	Caeca	13 "
64.	256 "	Caeca	11 "
65.	260 "	Caeca	12 "
66.	264 "	Caeca	11 "
67.	268 "	Caeca	13 "
68.	272 "	Caeca	12 "
69.	276 "	-	-
70.	280 "	Caeca	12 "
71.	284 "	Caeca	11 "
72.	288 "	-	-
73.	292 "	-	-
74.	296 "	Caeca	11 "
75.	300 "	Caeca	15 "
76.	304 "	Caeca	12 "
77.	308 "	Caeca	11 "
78.	312 "	Caeca	13 "

molt and typical adults were produced in 18-20 days. A period of 10 days was further needed for the developmental process culminating into mature adults.

Quite a good number of literature is available on the life cycle of Heterakis papillosa and Heterakis gallinarum which present little variation regarding the developmental processes. Riley and James (1921) found that complete development of the worm was attained by 24th day. Graybill (1921) observed that ova of Heterakis papillosa after hatching pass by way of the small and large intestine to the caeca where these undergo development to maturity. The entire life cycle from egg to adult required 64 days. Uribe (1922) recorded in the same worm that after hatching in the intestine completed its further development in the caeca. In no instance juveniles were found in other organs or other portion of the body. Adults found 56-61 days after ingestion of ova represented the complete duration of the life cycle. Dorman (1928) further reported that juveniles were recovered throughout the alimentary canal within 6 hours of the ingestion of Heterakis papillosa ova. Migration to the caeca took place in 17-98 hours and it took 36 hours to reach the adult stage.

Baker (1933) studied the development of Heterakis gallinarum in fowls and observed that the whole sequence is

completed within 30 days. Clapham (1933) reported that after ingestion of ova hatching took place within 6 hours. The juveniles reached directly to the caeca and mature there in the lumen.

Indications regarding the occurrence of a tissue phase are conflicting. There are authors who claim that the juveniles during the migratory course bore into or remain closely associated with the wall of the intestine and its glands. Riley and James (1921) reported that a few of the juveniles peneterated into the mucosa of caeca. Graybill (1921) also stated that although the juveniles were found only in the caeca after 3rd day, but in three instances these were found located in the wall of the same. Uribe (1922) recorded that most of the juveniles throughout the first stage of their development i.e., for the first five days were found burried in the glands of the caecal mucosa. As these increased in size many showed the anterior extremity inserted in the mucosa, but after the maturity was attained these were found free in the lumen of the caeca.

Contrary to these observations, Baker (1933) was not able to observe any close association of the worm, what-soever, with the glandular crypts of the mucosa. Based on the observations of Uribe (1922) that the larvae had a stage of

of parasitism within the tissues. Clapham (1933) observed that the sections obtained from the caeca and terminal portion of the intestine had no larvae in proximity of the walls of these organs.

The present study has revealed that during post mortem examination very few juveniles were recovered from the intestine and the caeca. Further, the juveniles with the completion of 168 hours were found disappeared from the caeca. It is believed that the juveniles have not followed the normal course of development as believed earlier, but after hatching entered into a tissue phase involving the mucosa and sub-mucosa layers of the intestine and caeca. These after completion of the 3rd molt move out again to occupy the lumen of the caeca. Although juveniles could not be located in the sections of the caeca but its wall presented degenerative changes in the superficial lininng of the epithelial cells and its glands (Fig. 82). Breach of muscularis mucosa has also been observed which showed that juveniles do enter into these parts or must have been in intimate association with these organs.

Tissue response:

Majority of the chickens to primary infection with this worm presented no clinical symptoms. The infected chicks

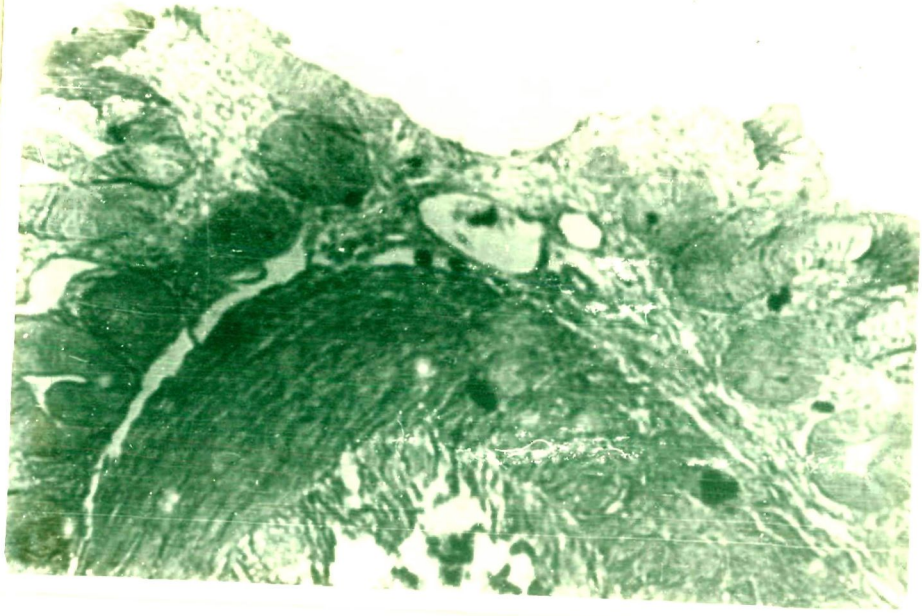


Fig. 82. Caecum primary infection, showing degenerative changes in muscularis mucosa associated with Heterakis gallinarum.

presented degenerative changes in the superficial lining of the epithelial cells and its glands (Fig. 83). Breach of muscularis mucosa has also been observed (Fig. 83). Few of the chickens were killed after 30 days and worms in large numbers were recovered. The caeca of these chickens revealed no deformity like invagination of the mucosa layers, thickened foci, rupture of the caecal wall and formation of something like nodules. The present author is against the views expressed by Itagaki (1930), Baker (1931), Spena (1935) and Clapham (1937) who have reported gross pathological changes and formation of nodule in the fowl during the course of primary infections. The author believes that the juveniles and the adults only make the tissue sensitive by their presence; and the cause of caecal lesions and formation of nodules are due to the repeated infections of the host with this worm. Studies on occurrence of caecal nodules in natural heterakiasis which follow now fully supports this assumption.

Caeca of fowls infected in nature apparently presented diseased patched and lesions on the surface. In some cases few areas of congestion have been observed. Microscopical examination of the sections prepared from the caeca were found to contain congestion of blood vessels distorsion of

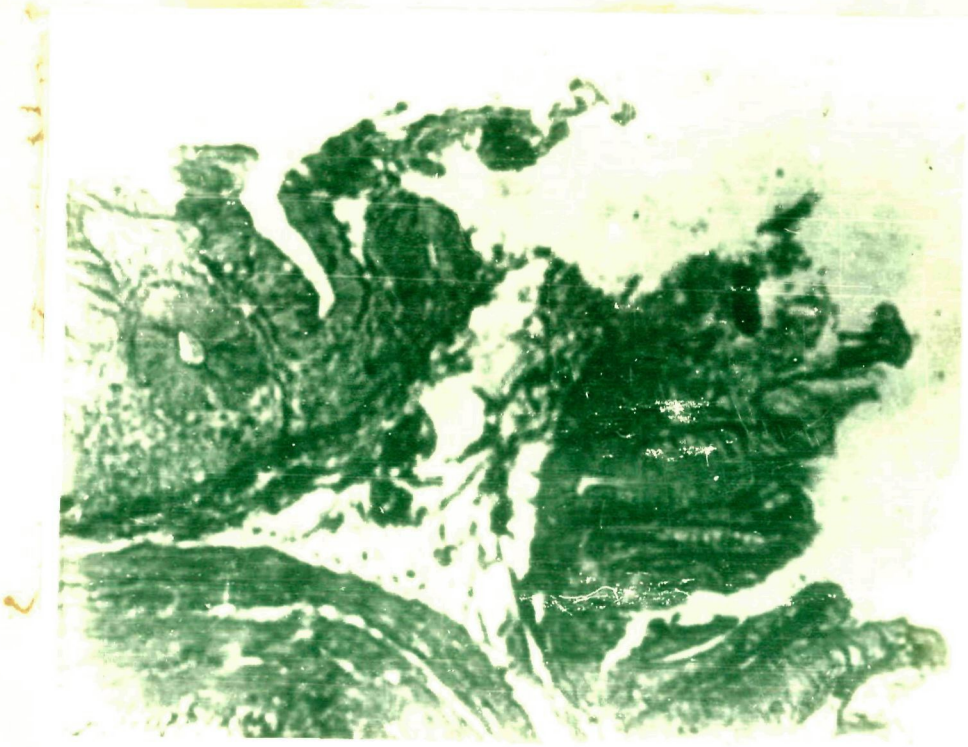


Fig. 83. Breach of the muscularis mucosa associated with Heterakis gallinarum.

the superficial lining of the epithelial cells (Fig.84). In heavily infected fowls where the caecal lesions were apparent, the formation of caecal nodule was very much evident. The worms were in close association with the mucosa, some embedding their heads in it, others entering into the nodules (Fig.85). The affected mucosa showed infiltration of lymphoid cells eosinophils, atrophy and necrosis of epithelial cells. The degeneration and desquamation of the superficial lining, are symptoms of Typhlitis as reported by Tyzzer (1934). Most of the lesions were found in the sections of the caecum below the attachment of the mesentery. Lesions accompanied by large number of nodules have been observed in the wall of both the caeca. The nodules were all defined, raised, circular and somewhat hard in texture. Nodules were not found in the caeca of normal fowls.

The sections at nodular portions showed well formed nodules in the sub-mucosa and some in the mucosa (Fig.86). From the musculature side these nodules were sometimes proliferated with muscularis mucosa fibres. At certain places there was heavy infiltration in the sub-mucosa together with many eosinophils and fibroblasts. Invagination of proliferated with muscularis mucosa fibres. At certain places there

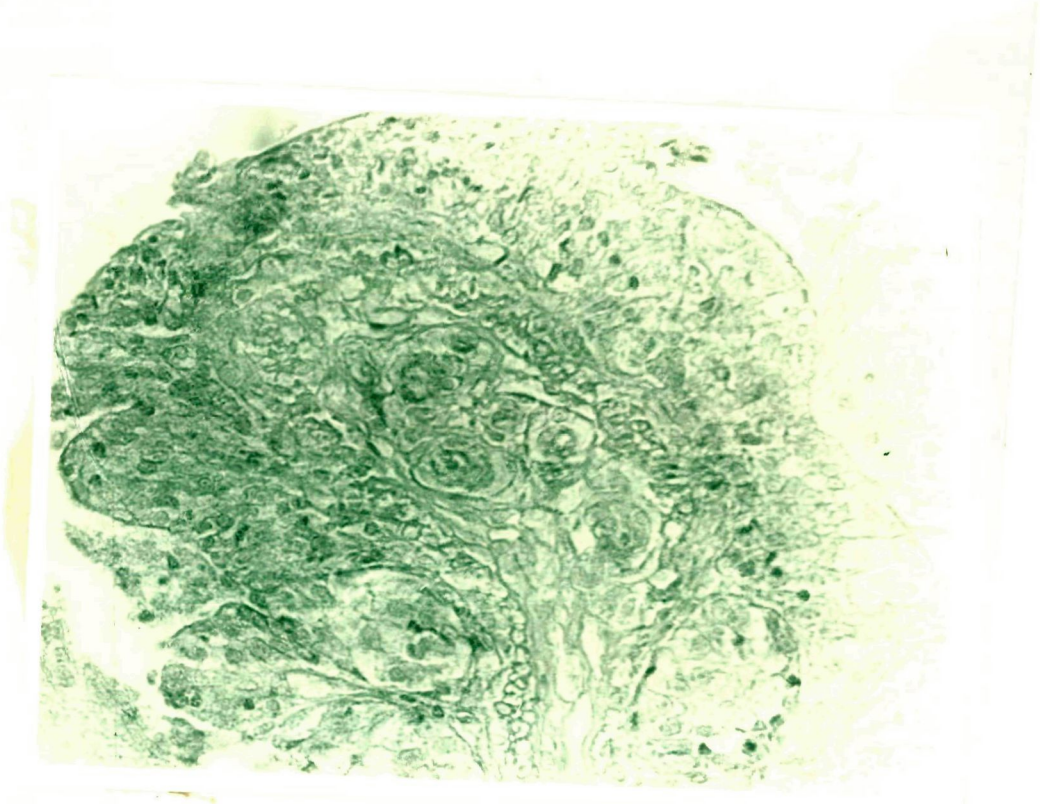


Fig. 84. Caecum infected in nature with Heterakis gallinarum showing distorsion of the superficial lining of the epithelial cells.

Fig. 85. Caecum infected in nature with Heterakis gallinarum showing close association of the worm with the mucosa, some embedding their head in it, others entering into the nodules.

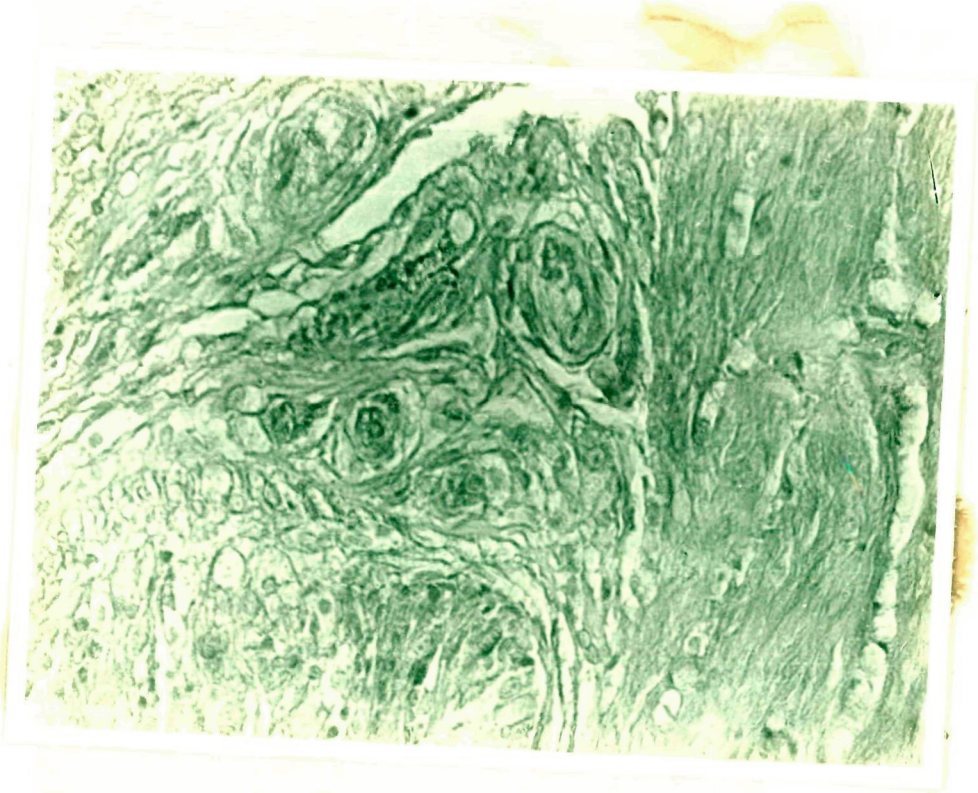


Fig. 86. Caecum infected in nature with Heterakis gallinarum, showing the lymphocytes eosinophils and fibroblasts, around sub-mucosal nodule, having young Heterakis gallinarum.

was heavy infiltration in the sub-mucosa together with many eosinophils and fibroblasts. Invagination of proliferated mucosa causing pressure atrophy of the musculature has also been observed. The presence of nodular lesions raised both serosal and mucosal surfaces.

Grigorev (1959) believed that perhaps some of adults enter in the already formed nodules and thus cause pressure atrophy and necrosis of the epithelial cells but the constitution of the nodules in the present study does not confirm the views expressed by Grigorev, as the nodules containing juveniles in the sub-mucosa, had no opening in the caecal lumen.

Kaushik and Sharma Deorani (1969) conducted experiments on the responses of Heterakis gallinarum infection in chickens. Their experiment with primary infection produced no result but nodules of varying sizes were observed in the caecal wall, only after subsequent infection of these chickens with the worm. The types of nodules observed, one associated with juveniles and the other with their absence. The author also concluded that the nodules were due to irritation caused by the juveniles and their metabolites, and that these were more a reaction to already sensitized

caeca due to subsequent infection. Their conclusion appears to be reasonable as they never observed any trace of nodule formation during the course of primary infection with this worm. The present study is further an elaboration of the facts presented by these workers on this aspect. The fowl in natural conditions receive repeated doses of infection and so the occurrence of nodule in their caeca has been a common feature. The ground is still open for further researches on the actual causes of formation of caecal nodules, whether it is because of the metabolites being released by the juveniles and the adults, or because of the already sensitized tissue of the host during its primary infection, or it is simply a manifestation of antibody antigen reaction, is still debatable.

Indirect life cycle:

Graybill (1921) expressed surprise that light infestation were obtained in the host through oral ingestion of Heterakis ova. He fed several hundred eggs to each chick, yet the number of worm in certain instances were very small. Dorman (1928) had also a similar experience and attributed this failure to the short duration of incubation. The author

also conducted experiment to infect chickens by oral ingestion of hatched juveniles which failed to produce any infestation. He emphasized the fact that a secondary host in which incubation and hatching of embryos might occur, is improbable, and so no intermediate host is necessary for completion of the life cycle. Ralliet and Lancet (1892) and Ackert (1917) succeeded in infesting chickens by feeding dung earthworm collected from a poultry yard. The latter author interpreted the result indicating that ova had apparently adhered to the worm or were carried within the body of earth worm, but did not believe the evidence and excluded the possibility that the earthworm might have acted in some way as an intermediate host.

A survey conducted by the present author on natural 'heterakiasis' indicated the involvement of some vector in its life cycle. The inference drawn was based on the data which presented a variation in the incidence of infection because the intensity of infestation reached to its highest peak in the rainy season and summer. Hence search for a probable vector prevalent during rainy season was carried out. Grasshoppers and earthworms which are most abundant during such periods were utilized in set of experiments

conducted on chickens for investigation. First batch of grasshoppers collected from the field failed to infuse any infection in the test chickens, and so was the case with grasshoppers collected nearby the poultry houses. But the grasshoppers reared in the laboratory infected with Heterakis gallinarum ova when fed to chickens gave positive results and worms were recovered from the caeca, though few in number i.e. one worm per bird. Earthworms collected from nearby poultry yard induced infection to such an extent that on an average 8.5 worms per bird were recovered from the caeca of the chickens. Comparatively light yield of adult worm (average 2.8 per bird) was obtained from the chickens fed with earthworms reared on filter paper induced infection. Recovery of worm was negligible approximately one worm per bird in the third category of earth worms collected from sources which had least possibility of the presence of Heterakis ova. Role of grasshoppers as a vector was least demonstrated in the present experiment. Earthworms in comparison to grasshoppers have shown the possibility of carrying infective eggs and may take an active tole in the transmission and propagation of caecal worm infection. Lund et al. (1958) have also enumerated the importance of earthworms to serve as true vector. They believed that the earthworms (i) served as a means of accumulation of embryonated

eggs in concentrated form because of its ability to migrate both laterally and vertically in the soil. These also (ii) protected the ova from the fungal activity and predation by other invertebrates in the soil.

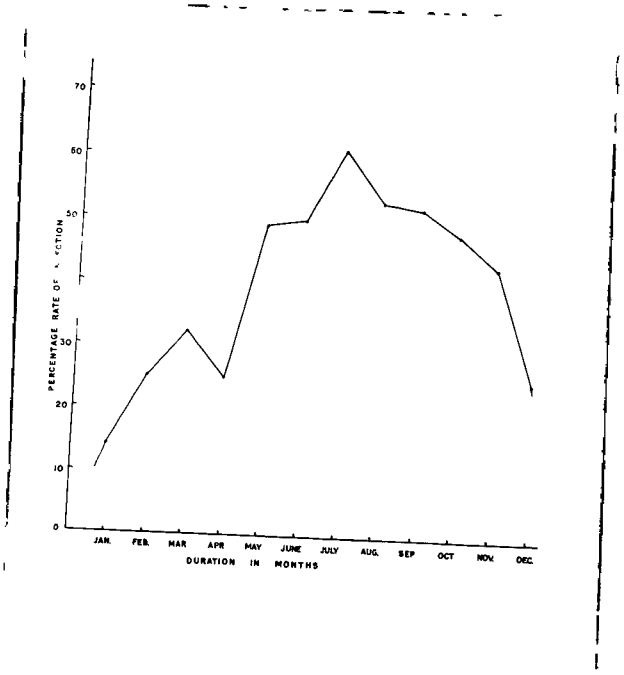
Incidence of infection and seasonal variation.

The results obtained have been shown in table 2 and 3, and represented by graphs A & B. It is evident from the study that high percentage of infection of this parasite was found during the months of July to September and the highest in the month of July with 61.33%. The lowest was found to be in the month of January, when only 14.18% infection was recorded.

The figures for Heterakis gallinarum infestation obtained in the present survey generally referred to adult birds. The birds which were examined had been in a variety of conditions. The source of individual bird was not traced, but these were received from a wide area around the city of Aligarh which included a number of neighbouring villages also. The conditions in which these fowls were reared were vastly different from one another. In village conditions very little attention, if any is paid to the food requirements of the fowls, daily in the morning they are allowed to go any where. The birds usually wander over long distances in search

Table 2. Frequency of infestation of Heterakis gallinarum in fowl.

Months	Frequency of infection			Percentage rate of infection
	Mild 1-20 worms	Moderate 21-40 worms	Heavy 41 - above	
January	90.00%	10.00%	Nil	14.18%
February	75.00%	25.00%	Nil	25.64%
March	86.95%	13.04%	Nil	32.39%
April	72.22%	11.11%	5.55%	25.00%
May	100.00%	Nil	Nil	49.31%
June	95.65%	4.34%	Nil	50.54%
July	89.13%	10.86%	Nil	61.33%
August	87.61%	10.76%	1.53%	53.71%
September	90.30%	80.60%	1.61%	52.10%
October	91.66%	8.33%	Nil	48.00%
November	95.45%	5.54%	Nil	43.42%
December	94.44%	5.55%	Nil	25.00%

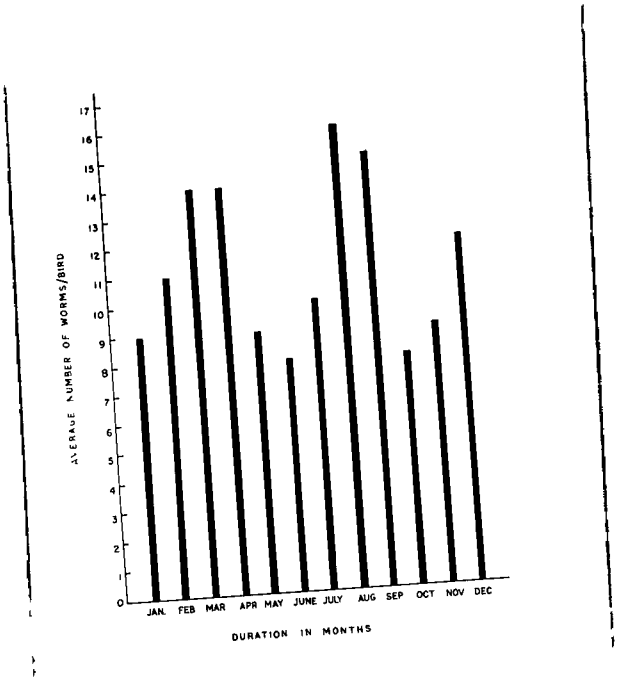


Graph A. Frequency of infection of Heterakis gallinarum in fowls.

of their food. There is usually no control over the disposal of waste matter which is done indiscriminately at the outskirts of villages and towns and popular forging sites of these fowls are rubbish grounds and refuse depots where they feed on remains of human food and faeces, carrion, insects, snails and earthworms etc.

Table 3. Seasonal incidence of Heterakis gallinarum infection in fowl.

Months	No. of fowls examined	No. of fowls infested	Total no. of worms recovered				Average no. of worms per infested bird
			Male	Female	Juvenile	Total	
January	74	10	31	60	0	91	9
February	78	20	87	136	4	227	11
March	71	23	133	193	5	331	14
April	76	18	98	161	3	262	14
May	73	36	128	208	8	344	9
June	91	46	150	258	4	412	8
July	75	46	166	277	19	462	10
August	121	65	423	676	4	1103	16
September	119	62	413	543	5	961	15
October	100	48	229	296	14	539	8
November	152	66	258	371	3	632	9
December	72	18	94	124	6	224	12



Graph B. Seasonal incidence of Heterakis gallinarum infection in fowl.

P A R T - C

C O N T R O L

The high rate of incidence of caecal nematode, Heterakis gallinarum, among fowls and its disease association has caused great economic loss to the country. It is estimated that more than 50% of the birds are lost annually due to this infestation. This big loss has made impossible the very idea of economic production. The problem of control of this parasite is assuming increasing importance. The most practical, and important control measure is the use of anthelmintics. A number of drugs were used and their efficacy was tested by exposing to experimentally infected chickens.

Experimental procedure

Heterakis gallinarum eggs were obtained by cutting up with fine scissors several freshly collected adult females. Most of the fertile eggs were settled at the bottom of the dish leaving a great bulk of the infertile eggs and worm debris in suspension. These were later eliminated by siphoning off the supernatant fluid. The sediment containing fertile eggs was kept in an incubator at 27 $\pm$ 1 ^{o}C for 17 days to achieve embryonation. Only cultures less than 6 weeks old were used to infect chickens.

The chickens infected with embryonated eggs were at the age of 3 to 4 weeks. Each bird received in single

infection about 600 eggs as a standard dose. Each bird of the experimental group and of the control group was fed with ova alternately in order to minimise possible difference in the number of eggs received from the stock solutions.

These birds found positive with infection were isolated and kept on wire floor cages for a week in advance in order to allow them to get accustomed to the new feeding environment. The birds were later treated with the following drugs in order to test the comparative efficacy against Heterakis gallinarum infection.

1. Phenothiazine
2. Piperazine adipate
3. Enheptin - T
4. Resorcinol
5. Carbon tetrachloride
6. Tetrachloroethyline
7. Carbon disulphide

The drugs to the chickens were given orally. Solid form of the drugs were given as food pellets at the rate of 100, 200, and 300 mg/kg body weight, and those in liquid form as drops at the rate of 1 ml, 2 ml and 3 ml per kg body weight and various dosage were spread over at 10 A.M., 2 P.M., 6 P.M. and 10 P.M. during the period of 24 hours.

Effect of medication on the adult worm was evaluated by examination of the faeces and necropsy of the treated birds. Following 24 hours and 72 hours of the treatment adult worms passed by a bird was recorded. The birds were sacrificed later small intestine and caeca were removed. These organs were cut open longitudinally and worms were recovered. Efficacy of the drugs on adult worms was determined by estimating the per cent reduction of the parasite in the intestine and caeca of the treated birds as compared with those recovered from the control birds at necropsy.

Results and Discussion

Summary of the results obtained are recorded in Tables 4 to 9 and presented by the Graph C. Phenothiazine effected the elemination of adult worms at all doses. The initial dose of 100 mg / kg body weight eliminated 4 out of a total of 23 worms showing efficacy of 20.5% (Table 4). Therapeutic activity of the drug increased with the increase in the dosage and approximately ED_{50} was obtained at the highest dose of 300 mg/kg. Anthelmintic activity of the drug was more elaborated in those experiments in which treated chickens were kept for 72 hours prior to necropsy. This period was sufficient enough to remove about 75% of the worms from the infected chickens. The dose appeared

to be reasonably safe and useful. The results also indicated that the efficacy of this drug was correlated with the dose and that 300 mg/kg approached the optimum level. The drug in small multiple doses is particularly active in suppressing egg production, and the effect of drug may be accumulative for susceptible nematodes. The only draw back that the drug is easily excreted by the host.

McCulloch and Nicholson (1940) found 50 mg and 500 mg as satisfactory individual dose. The average effectiveness ranged from 95 - 100%. About 500 times the smallest effective dose had no harmful effect on the chickens, but these massive doses showed no anthelmintic action.

The efficacy of phenothiazine as anthelmintic depends very much upon the location of the parasite in the digestive tract. It was greater in caecum and colon region than the small intestine where mucosa is more specialized for absorption. Harwood and Guthrie (1944) have shown that the drug is partially effective in the removal of Ascarids, but Heterakids indicated high susceptibility to this medication. It is obvious that the drug has a differential affinity for certain nematodes.

The drug although incapable of supressing the growth and development of the parasite, but Wehr and Olivier (1946) reported that the drug was quite capable of forcing the worms to be expelled out soon after reaching maturity.

The other test drug, piperazine adipate was administered at the dose rate of 100, 200 and 300 mg/kg body weight spread over a period of 24 hours. Number of worms passed out and worms recovered at necropsy of the chickens after post treatment period of 24 hours and 72 hours are presented in Tables 4 to 6. The drug showed mild effect as on an average 13.6, 24.3 and 34.5% efficacy was obtained at different dosage level. The drug administered at 300 mg/kg which produced 21.4 and 47.6% efficacy during 24 hoursand 72 hours post treatment period was the only satisfactory result. Larson and Hansen (1957) studied the chemoprophylactic action of piperazine dihydrochloride in chickens and observed that total dosage above 500 mg/kg did not reduce the number of adult or larval Heterakis gallinarum. Shumard and Eveleth (1955) believed that the drug is more effective against Ascaridia galli in fowls, but less so against Heterakis gallinarum.

Test carried out on experimentally infected chickens with Enheptin-T medicated food provided not much promising results as complete protection could not be obtained. Chickens given the dose at the rate of 100, 200 and 300 mg/kg body weight passed out only 2-5 worms out of the total of approximately 20-25 worms recovered at necropsy (Table 4 to 6). The drug was partially effective at the dose rate of 300 mg/kg with the post treatment period maintained for about 72 hours

as it gave on an average 18.4% efficacy. However, Horton-Smith and Long (1951) in a series of experimentally infected poults with Heterakis gallinarum have found Enheptin-T as the most effective compound. A mash containing 0.05% drug first fed on the 4th day after infection gave complete protection while 0.1% proved excellent in the treatment of established infection. Swales (1952) also confirmed anthelmintic activity of this drug on the larval stages of H. gallinarum.

Other drugs tested to demonstrate the anthelmintic activity were found to be ineffective except carbon tetrachloride. An ED_{50} was obtained with the use of single oral dose of carbon tetrachloride at the dose rate of 3 ml/kg after the post treatment duration of 72 hours. Other compounds (with maximum doses given in parenthesis) tested were resorcinol (300 mg/kg), tetrachloroethylene (3 ml/kg), carbon disulphide (3 ml/kg) gave 10.5%, 16.6% and 12.4% efficacy respectively. These results demonstrated the complete failure of the drugs against Heterakis gallinarum infection, and in some cases these have shown to produce some unhealthy side effects. It was observed that increase in the maximum tolerated dose of tetrachloroethylene, carbon disulphide and resorcinol may even cause the death of chickens. But in case of phenothiazine, piperazine adipate, Enheptin-T and carbon tetrachloride no side effects were observed.

Table 4. Comparative efficacy of the drugs at dose rate 100 mg/kg on adult Heterakis gallinarum

Drugs	Post treatment duration	Weight of chickens in gms	Dose in mg	Worms passed	Worms recovered	Per cent efficacy	Average (%)
Phenothiazine	24 hrs	378	3.78	4	19	17.3	20.0
	72 hrs	431	4.31	5	17	22.7	
Piperazine adipate	24 hrs	385	3.8	3	17	15.1	13.6
	72 hrs	342	3.4	2	15	11.7	
Enheptin - T	24 hrs	215	2.1	2	21	8.6	7.6
	72 hrs	250	2.5	2	28	6.6	
Resorcinol	24 hrs	257	2.5	2	29	6.4	5.5
	72 hrs	254	2.5	1	20	4.7	

Table 5. Comparative efficacy of the drug at the dose rate 200 mg/kg on adult Heterakis gallinarum.

Drugs	Post treatment duration	Weight of chickens in gms	Dose in mg	Worms passed	Worms recovered	Per cent efficacy	Average (%)
Phenothiazine	24 hrs	404	8.0	7	10	41.1	46.4
	72 hrs	272	5.4	14	13	51.8	
Piperazine adipate	24 hrs	300	6.0	6	22	21.4	24.3
	72 hrs	375	6.1	6	16	27.2	
Enheptin - T	24 hrs	185	3.7	2	11	15.3	13.2
	72 hrs	302	6.0	2	16	11.1	
Resorcinol	24 hrs	498	9.9	1	13	7.1	9.3
	72 hrs	304	6.9	3	23	11.5	

Table 6. Comparative efficacy of the drugs at dose rate 300 mg/kg on adult Heterakis gallinarum.

Drugs	Post treatment duration	Weight of chickens in gms	Dose in mg	Worms passed	Worms recovered	Per cent efficacy	Average (%)
Phenothiazine	24 hrs	326	9.7	11	11	50.0	62.5
	72 hrs	304	8.8	12	4	75.0	
Piperazine adipate	24 hrs	434	12.8	6	22	21.4	34.5
	72 hrs	323	9.6	10	11	47.6	
Enheptin - T	24 hrs	307	9.2	4	18	18.1	18.4
	72 hrs	273	8.1	3	13	18.7	
Resorcinol	24 hrs	345	10.3	4	25	13.7	10.5
	72 hrs	233	6.9	2	25	7.4	

Table 7. Comparative efficacy of the drugs at the dose rate 1 ml/kg on adult *Heterakis gallinarum*.

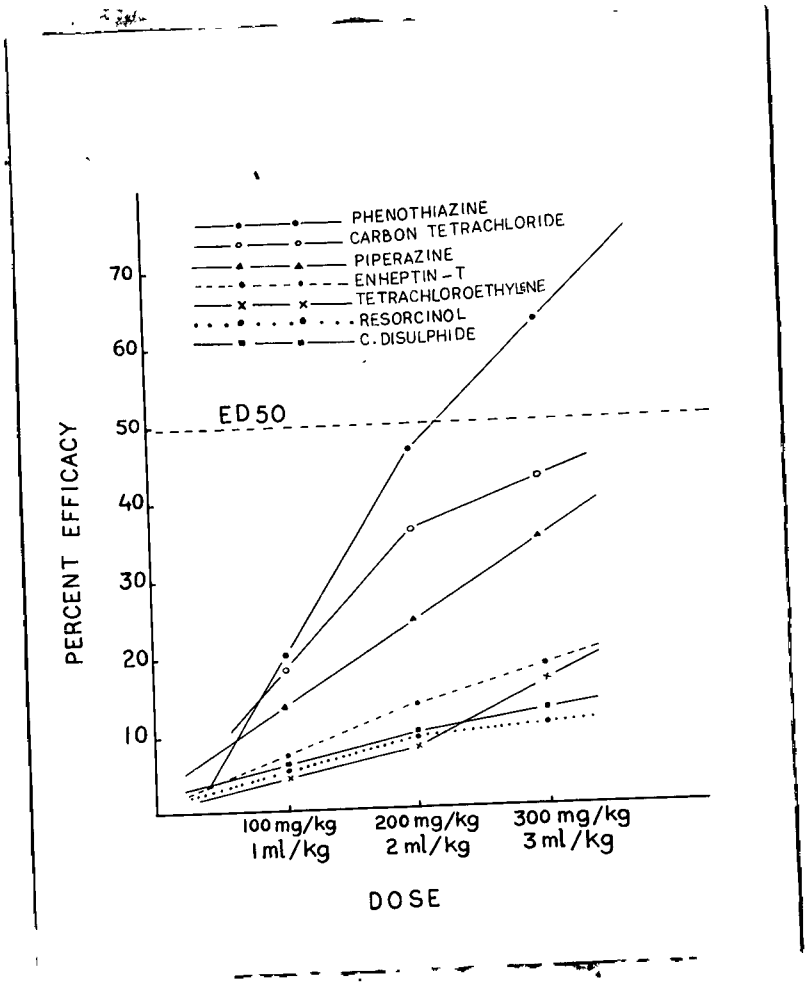
Drugs	Post treatment duration	Weight of chickens in gms	Dose in ml	Worms passed	Worms recovered	Per cent efficacy	Average (%)
Carbon tetrachloride	24 hrs	275	0.27	3	14	17.6	18.4
	72 hrs	300	0.3	5	21	19.2	
Tetrachloroethylene	24 hrs	275	0.27	1	30	3.2	4.7
	72 hrs	300	0.30	1	15	6.2	
Carbon disulphide	24 hrs	300	0.3	1	20	4.8	5.1
	72 hrs	275	0.27	2	29	6.4	

Table 8. Comparative efficacy of the drugs at the dose rate 2 ml/kg on adult *Heterakis gallinarum*.

Drugs	Post treatment duration	Weight of chickens in gms	Dose in ml	Worms passed	Worms recovered	Per cent efficacy	Average (%)
Carbon tetrachloride	24 hrs	375	0.7	5	14	26.3	36.2
	72 hrs	375	0.7	12	14	46.1	
Tetrachloroethylene	24 hrs	350	0.7	1	22	4.3	8.4
	72 hrs	375	0.7	3	21	12.5	
Carbon disulphide	24 hrs	350	0.7	2	20	9.0	9.5
	72 hrs	350	0.7	3	26	10.0	

Table 9. Comparative efficacy of the drugs at the dose rate 3 ml/kg on adult *Heterakis gallinarum*.

Drugs	Post treatment duration	Weight of chickens in gms	Dose in ml	Worms passed	Worms recovered	Per cent efficacy	Average (%)
Carbon tetrachloride	24 hrs	400	1.2	6	15	28.5	42.2
	72 hrs	400	1.2	14	11	56.0	
Tetrachloroethylene	24 hrs	400	1.2	3	14	17.6	16.6
	72 hrs	300	0.9	5	27	15.6	
Carbon disulphide	24 hrs	500	1.2	3	24	11.1	12.4
	72 hrs	500	1.2	4	25	13.7	



Graph C. Per-cent efficacy of different drugs at various doses on Heterakis gallinarum.

SUMMARY

A detailed study on the histological anatomy of the worm has been carried out by preparing serial sections of the worm through paraffin microtechnique. Body wall has been found to be constituted of three distinct components - cuticula, sub-cuticula and musculature. The cuticula has been found to contain five different layers. The sub-cuticula is a syncytial layer lying below the cuticula. It bulges into the pseudocoel at four regions of the body in the form of ridges which are known as dorsal, ventral and lateral chords. The chords undergo modifications at the regions of the nerve-ring, excretory pore, vulva and anus. The chords also serve to hold in them the longitudinal nerves and the lateral excretory canals. The musculature consists of a single layer of more or less spindle-shaped muscle cells attached to the sub-cuticula throughout the entire length of the body. The musculature is of the polymyarian and coelomyarian type. Because of the longitudinal chords the musculature has been found to be thrown into four sectors.

The digestive system in Heterakis gallinarum consists of a simple and straight tube extending throughout the body

from the anterior mouth opening to terminate in the posterior part on the ventral side of the body. The mouth opening is rounded and encircled by three lips, two latero-ventral and one dorsal in position, and each of the lip is provided with two cephalic papillae. The mouth via a short stoma opens into the oesophagus which is tubular in the beginning but terminates into a bulb. The lumen of the oesophagus is tri-radiate and the end bulb is provided with a valve. The oesophagus is covered over by a layer called tunica propria and the ground tissue is comprised of marginal and radial muscle which also enclosed in them the oesophageal glands and the nerve tissue from the sympathetic nervous system. The intestine is a straight tube, the wall of which is composed of epithelial cells. It has been divided into three regions; anterior part or the ventricular region, mid-part or the intestine proper and posterior part or the pre-rectal region. The intestine communicates further into the rectum via the intestino-rectal valve. The rectum is a short irregular flattened tube lined inside by the general body cuticle. It also receives the opening of the rectal glands near its junction with the intestine. In females the rectum forms an exit through the anal pore on the ventral side, but in males a common ano-genital passage, the cloaca is present which results in the modification of the rectum.

The excretory system is that of simple H-type and comprised of an excretory pore, terminal excretory canals. The excretory pore is situated in the anterior part of the body shortly behind the nerve-ring. Internally it leads into the terminal excretory duct, which passes through the tissues of the ventral nerve chord, first in the antero-dorsal direction, then bends backwards and opens into the excretory sinus. The sinus via transverse canal, forms communication with the lateral excretory canals.

The main part of the nervous system is the nerve ring, various ganglia and nerves associated with them. From the nerve ring six cephalic papillary nerves; 2 sub-dorsal, 2 sub-ventral and 2 lateral ascend upwards to the head end and terminate at the base of the cephalic papillae of the respective lips. Associated with the nerve-ring are found four cephalic ganglia - dorsal, ventral and a pair of lateral ganglia being embeded in the respective chords. Amphids receive nerves which originate from the amphidial ganglia present in close opposition to the lateral ganglia. Four main nerves , the so called somatic nerves originate from the respective ganglia and proceed posteriorly in the chords till the hinder part of the body. The dorsal somatic nerve terminates insignificantly, but the ventral nerve form the pre-anal

ganglion and terminates into the anal ganglion close to the anus. The lateral somatic nerves form the lumbar ganglion in the caudal region and terminate into the phasmids. In males five pair of nerves arise from lateral somatic nerves and innervate the genital papillae and the sucker.

Female reproductive system comprised of vulva, vagina, uterus, oviduct and ovary. The worm falls in the didelphic and amphidelphous group. Each of the ovary is composed of an epithelial layer and a germinal chord, the blind end of it is covered by a small cell known as the cap cell. The ovary is divided into germinal zone and growth zone, containing germ cells in different stages of development. Ovary communicates into the oviduct which has got two layers in its wall, the outer membranous layer and the inner epithelial layer. The oviduct running for a short distance opens into the uterus is a thin walled wide tube which occupies most of the available space in the body. The two uteri join and communicate into the vagina. The vagina is a narrow thick wall and highly muscular tube, the terminal part of it is termed as the ovijector. The vulva is in the form of a transverse slit and is equatorial .

The male reproductive system is more or less in the form of a straight tube. It begins with a single irregular

testis present in the anterior part of the body, which is continued after a sharp constriction into a small seminal reservoir. The latter narrows towards its distal end and forms the vas deferens which becomes highly muscular in its distal part and opens into the cloaca. There are a number of accessory organs associated with the male genital apparatus. These include the caudal alae, caudal papillae two unequal spicules and the sucker.

Preliminary observations on the life cycle of the worm has also been undertaken. This comprised of the study of the embryonic development upto the infective stage. Different media were tested for cultivation of ova at different temperatures. In media containing distilled water and 1% formalin, first division of the single celled stage took place after 21 hours. During the first stage of the cleavage the egg appeared exceedingly granular, and the nuclei of the two resulting blastomeres were peripherally located. In most of the cases the resulting blastomeres divided simultaneously, quickly followed by further divisions forming a morula in three days. The activity continue for five days and in the mean time a depression formed between the two types of blastomeres and the embryo grew terminally transforming it into a vermiform embryo. The whole process was completed in 13 days.

In normal saline first division occurred in 24 hours, morula stage was produced in 4 days and complete development was obtained in 15 days. The development in potassium dichromate was slow and the whole sequence completed in 17 days. Development in nitric acid was quite rapid and took only 16 days for its completion.

Post embryonic development and migratory course in vivo was made by feeding embryonated ova to groups of parasite free chickens reared in the laboratory. Hatching of the ova took place in the proximal portion of the intestine in 2-3 hours, and second state juveniles reached the caeca within 168 hours. The juveniles instead of undergoing normal development in the lumen of the caeca as believed earlier, entered into a tissue phase involving the mucosa and sub-mucosa layers. After the completion of the third molt, the fourth stage juveniles appeared again in the lumen of the caeca within 224 hours of the initial infection. During the 28 - 30 hours these passed through the fourth molt and typical adults were produced in 18 - 20 days. The whole developmental process within the host, was completed in 29 - 31 days.

The sections of the caeca taken from chickens during the course of their primary infection presented degenerative

changes in the superficial lining of the epithelial cells and its glands. Breach of muscularis mucosa has also been observed. Studies on natural "heterakiasis" was also made and the fowls infected in natural conditions presented diseased patches and lesions on the surface of the caeca. The sections showed well formed nodules in the sub-mucosa and some in the mucosa. At certain places there had been heavy infiltration in the sub-mucosa together with many eosinophils and fibroblasts. Invagination of the proliferated mucosa causing pressure atrophy of the musculature has also been observed.

Heterakis gallinarum is believed to have a direct development. Search for a probable vector was also carried out. Earthworms and grasshoppers which are most abundant during rainy season were utilised in set of experiments on chickens conducted for investigation. The results obtained presented a positive role of earthworms as vector in the transmission of infection.

Studies on the incidence of the worm among fowl population of Aligarh District has been conducted for a year. The results obtained show seasonal variation in the incidence of infection, the highest percentage was found

during the months of July and August (rainy season) the lowest in December and January (dry season).

Chemotherapeutic measures agains Heterakis gallinarum were also undertaken by using different anthelmintics drugs. Screening of various chemical to be used as anthelmintic was made. Different doses of the drugs were administered and efficacy was studied by exposing it to the infected chickens. A comparative efficacy of these chemicals based on the worms eleminated in the faeces and recovery of adult worms on necropsy was worked out. It was observed that phenothiazine is the most effective drug against Heterakis gallinarum, while piparazine adipate and Enheptin-T comes next and to some extent carbon tetrachloride was also effective. Rest of the drugs, i.e. resorcinol, tetrachloroethylene and carbon disulphide were quite effective. In some cases, these were found to produce some unhealthy side-effects and may even cause death of the chicken.

REFERENCES

Ackert, J.E. 1917. A means of transmitting the fowl nematode, Heterakis papillosa Bloch. Science 46: 394.

Ackert, J.E. 1931. The morphology and life-history of the fowl nematode, Ascaridia lineata (Schneider). Parasit., 23: 360-379.

Ansari, J.A. and Basir, M.A. 1965. The histological anatomy of Setaria cervi (Rudolphi, 1819). Ind. J. Helm. 15, Monographic Suppl.

Apathy, S. 1893. Ueber die Muskelfaseru von Ascaris nebst Bemerkungen uber die von Lumbricus und. Hirudo. Ztschr. Wiss. Mikr. 10: 36-73.

Apathy, S. 1894. Dus leitende Element in den Muskefaseru von Ascaris. Arch. Mikr. Anat., 43: 886-911.

Baker, A.D. 1931. Some pathological conditions found in the caeca of fowls. Poult. Sci. 46: 394.

Baker, A.D. 1933. Some observations on the development of the caecal worm, Heterakis gallinae (Gmelin, 1790) Freeborn, 1923, in the domestic fowl. Sci. Agric. 13: 356-363.

Baker, A.D. 1936. Studies on Heterakis gallinae (Gmelin, 1790) Freeborn, 1923, a nematode parasite of domestic fowls. Trans. Roy. Canad. Inst. 20: 179-215, 21: 51-86.

Bastian, H.C. 1866. On the anatomy and physiology of the nematode, parasitic and free, with observations on their zoological position and affinities to the echinoderms. Phil. Trans. Roy. Soc. Lond., 156: 545-638.

Beach, J.R. and Freeborn, B. 1930. Diseases and parasites of poultry in California. Circ. agric. Ext. Serv., Univ. Calif. 8. cf. Aust. J. exp. Biol. med. Sci. 1937. 15: 429-439.

Chitwood, B.G. 1929. Notes on the copulatory sac of Rhabditis strongyloides Schn. Helm. Soc. Wash. J. Parasit., 15: 281-292.

Chitwood, B.G. 1930. Studies on some physiological functions and morphological character of Rhabditis (Rhabditidae, Nematodes). J. Morph. Physiol. 49: 251-275.

Chitwood, B.G. 1931. A comparative histological study of certain nematodes. Ztschr. Morph. 23: 237-284.

Chitwood, B.G. 1932. The basic characters of nervous system of nematodes. J. Parasit. 19: 167.

Chitwood, B.G. and Hill, C.H. 1932. A note on the oesophageal glands of Ascaris lumbricoides. Ztschr. Zellforsh. 14: 605-615.

Chitwood, B.G. and Chitwood, M.B. 1950. An introduction to Nematology Sec. 1 Ana., Baltimore, Md.

Christensen, R.O. 1940. Studies on some early developmental stages of nematode worms. J. Tennessee Acad. Sci. (Abst.) 15: 416.

Christensen, R.O. and Creel, H.H. 1942. Soil temperature and soil moisture as factors in the seasonal incidence of certain animal parasites in Albama. J. Albama Acad. Sci. 14: 30-33.

Christensen, R.O., Earle, H.H. Jr., Butler, R.L. Jr. and Creel, H.H. 1942. Studies on the eggs of Ascaridia galli and Heterakis gallinae. Trans. Amer. micr. Soc. 61: 191-205.

Clapham, P.A. 1933b. On the life-history of Heterakis gallinae. J. Helm. 11: 67-86.

Clapham, P.A. 1934a. Some observations on the response of chickens to infestation with Heterakis gallinae. Ibid. 12: 71-78.

Clapham, P.A. 1938. The relation of Helminthiasis to Leukaemia in domestic fowls. Ibid. 16: 52-56.

Cobb, N.A. 1923. Interesting features in the anatomy of nemas. J. Parasit., 9: 242-243.

Dauglieva, E.K. 1966. Study of some biochemical changes in the blood in Heterakis infections of chickens. Konferntsiya Biokhimikov Respublik Srednei Azii i Kazakhstana, Alma. Ata(ist), 223-224 (In Russian).

Dauglieva, E.K. 1966. Maturation and survival of Heterakis eggs in the open. Vest. sel-Khoz. Nanki. Alma-Ata., 10: 61-65 (In Russian).

Deineka, D. 1908. Das Nervensystem von Ascaris. Ztschr. Wiss. Zool. 89: 242-307.

Dorman, H.P. 1928. Studies on the life cycle of Heterakis papillosa (Bloch.). Trans. Amer. micr. Soc. 47: 279-313.

Dujardin, F. 1845. Histoire naturelle des Helminthes ou Vers intestinaux. 222-229.

Eberth, C.J. 1860. Die Generation sorgane von Trichocephalus dispar. Ztschr. Wiss. Zool. 10: 383-400.

Fedotova, M.N. 1969. Hygromycin B as an anthelmintic against ascaridiasis and heterakiasis in chickens. Byull. Vses. Inset. Gelomint. K. I. Skrjabina. No. 2 · 106. 109.

Fernando, S.D.A., Jayasinghe, J.B. 1963. Methyridine as an anthelmintic for Heterakis spp. and Capillaria spp. in domestic gees. Ceylon vet. J. 11: 97-98.

Frank, J.F. 1953. A note on the experimental transmission on entero hepatitis of turkeys by arthropods. Canad. J. Comp. Med. 17: 230-231.

Freeborn, S.B. 1923. Nicotine as a poultry vermifuge. Science 57: 692-693.

Froelich, F. 1791. Beitrage zur Naturgeschichte der Einegeweiderwurmer. Naturforschr. Halle. 25: 52.

Galli-Valerio, F. 1898. Le neoformatzioni nodulari Manuale di Patagonia generale comparata e sperimentale, Milano, 110. cf. Clapham, P.A. 1933. J. Helm. 11: 67-86.

Gmelin, J. Fr. 1790. Caroli a Linne-Systema Naturae per regna trianaturae secundum classes ordines, genera, locis. Vol. I. Editio decima tertia, sucta, reformata, cure Jo. Fred. Gmelin Pt. 6 (Vermes): 3,021-3,910. cf. Clapham, P.A. 1933. J. Helm. 11: 67-86.

Goeze, J.A.E. 1782. Naturgeschichte 86 pp. cf. Clapham, P.A. 1933. J. Helm. 11: 67-86.

Graybill, H.W. and Smith, T. 1920. Production of fatal blackhead in turkeys by feeding embryonated eggs of Heterakis papillosa. J. Exp. Med. 31: 647-655.

Graybill, H.W. 1921. Data on the development of Heterakis papillosa in the fowl. J. Exp. Med. 34: 259-270.

Goldschmidt, R. 1905. Uber die cuticula von Ascaris. Zool. Anz. 28: 259-266.

Goldschmidt, R. 1908a. Das Nervensystem von Ascaris lumbricoides und megalocephala 1. Ztschr. Wiss. Zool. 90: 73-136.

Goldschmidt, R. 1910. Das Nervensystem von Ascaris lumbricoides und megalocephala III. Teil. Festschrift. R. Hertwig. 2: 255-354.

Grigorev, N.K. 1959. The role of Heterakis gallinae in the formation of nodule in the caecum of domestic fowls. Trudy. mosk. vet. Akad., 25: 237-244.

Grigorev, N.K. 1959a. Pathological changes in the caecum and liver of domestic fowls infected with Heterakis. Ibid. 25: 245-254.

Guthrie, J.E. and Harwood, P.D. 1942. The efficacy of phenothiazine and nicotine bentonite for the removal of Heterakis gallinae and Ascaridia galli from chickens. J. Parasit. 28: 224-225 (Suppl.).

Harwood, P.D. and Guthrie, J.E. 1944. Effect of nicotine bentonite and certain physical state upon the efficacy of phenothiazine against nematodes in fowls. Expl. Parasit. 30: 143-152.

Hesse, R. 1892. Ueber des Nervensystem von Ascaris megalocephala. Diss. Halle Also in Ztschr. Wiss. Zool. 54: 548-568.

Hetherington, D.C. 1923. Comparative studies on certain features of nematodes and their significance III. Biol. Monogr. 8: 1-62.

Hodasi, J.K.M. 1969. Comparative studies of Helminth fauna of native and introduced domestic fowls in Ghana. J. Helm. 43: 35-52.

Horton-Smith, C. and Long, P.L. 1951. Enheptin-T in the treatment of Histomoniasis (Blackhead) in turkeys. The Vet. Rec. 63: 507.

Immink, B.D.C.M. 1924. On the microscopical anatomy of the digestive system of Strongylus edentatus Looss. Arch. Anat. Hist. Embryol. 3: 281-326.

Itagaki, S. 1930. The nature of parasitic nodules in the caecal wall of fowls and the development of Heterakis vesicularis. Proc. 4th. World's Poult. Congr. 481-483.

Jaegerskioeld, L.A. 1894. Beitrage zur Kenntni der Nematoden. Zool. Jahrb., Abt. Anat. 7: 449-532.

Johnson, S. 1968. The histological anatomy and life-history of Physaloptera varani Parona, 1889 (Nematoda: Spiruroidea). Ind. J. Helm. 20: 1-83 (Suppl.).

Kaushik, R.K. and Sharma Deorani, V.P. 1969. Studies on the tissue response in primary and subsequent infections with Heterakis gallinae in chicken and on the process of formation of caecal nodules. J. Helm. 43: 69-78.

Larson, I.W. and Hansen, M.F. 1957. Chemoprophylactic action of piperazine dihydrochloride against a typhlitis in chickens. J. Parasit. 43: 19-20.

Letulle, M. and Marotel, G. 1909. Etudes des typhlitis parasitaire nodules des caecums parasitaires chez le Faisan. Arch. Parasit. 12. cf. Clapham, P.A. 1933. J. Helm. 11: 67-86.

Leuckart, K.G.F.R. 1876. Die Menachlichen Parasiten., 2: 513-882, 2: 368-369, Leipzig.

Looss, A. 1905. The anatomy and life history of Ancylostoma duodenale. Rec. Egypt. Govt. Schl. Med. 3: 1-158.

Lund, E.E. 1958. Growth and development of Heterakis gallinae in turkeys and chickens infected with Histomonas meleagridis. J. Parasit. 44: 297-301.

Lund, E.E. and Burtner, R.H., Jr. 1958. Effect of four embryonation media on the embryonation and infectivity to chickens of Histomonas bearing eggs of Heterakis. Ibid. 44: 197-200.

Lund, E.E. 1960. Factors influencing the survival of Heterakis and Histomonas on soil. Ibid. 46: 38 (Suppl.).

Lund, E.E. and Ellis, D.J. 1963. Role of earthworms in transmission of Heterakis and Histomonas to turkeys and chickens. Ibid. 49: 50. (Suppl.)

Lund, E.E., Wehr, E.E. and Ellis, D.J. 1966. Earthworm transmission of Heterakis and Histomonas to turkeys and chickens. Ibid. 52: 899-902.

Madsen, H. 1949. Heterakis gallinarum (Schrank, 1788) nec. Heterakis gallinae (Gmelin, 1790). Ibid. 35: 543.

Madsen, H. 1962. The so called tissue phase in nematodes. J. Helm. 36 : 143-148.

Magath, T.B. 1919. Camallanus americanus nov. spec. Trans. Amer. Micros. Soc. 38: 49-170.

Man, J.G. de. 1886. Anatomische Untersuchungen uber freilebende Norsee Nematoden. 82 pp.

Martini, E. 1808. Die Konstanz histologischer Elemente bei Nematoden nach Abschluss der Entwickelungsperiode. Verkandl. Anat. Geselisch. 22: 132-136.

Martini, E. 1912. Bemerkungen uber den Bau der Oxyuren. Zool. Anz., 39: 49-53.

McCulloch, E.C. and Nicholson, L.G. 1940. Phenothiazine for the removal of Heterakis gallinae from chickens. Vet. Med. 35: 398-400.

Meads, E.B. and Taylor, P.A. 1963. Typhlitis in pheasants due to Heterakis gallinae. Canad. Vet. J. 4: 56-59 (French Summary).

Monnig, H.O. 1927. The life history of Trichostrongylus instabilis and Trichostrongylus rugatus of the sheep in South Africa. Rep. Vet. Sci. Africa 11 and 12: 231-251.

Morgan, D.O. and Wilson, J.E. 1938. Observations on helminth parasite of poulty in Scotland. J. Helm. 16: 165-172.

Morgan, D.O. and Wilson, E.J. 1939. The occurrence of Heterakis gallinae in poultry and its relation to disease breed and other helminths. Ibid. 17: 177-182.

Muller, J.F. 1929. Studies on the microscopical anatomy and physiology of Ascaris lumbricoides and Ascaris megalocephala. Ztschr. Zellforsch. 8: 395-396.

Musso, R. 1930. Die Genitalrohren von Ascaris lumbricoides und megalocephala. Ztschr. Wiss. Zool. 137: 274-363.

Nath, D. and Pande, B.P. 1963. Lesions associated with some nematode parasites in the alimentary canal of Indian domestic fowls. Res. Vet. Sci. 4: 390-396.

Norton, C.C. 1964. A survey of nematodes from the lower digestive tract of domestic fowls. J. Helm. 38: 269-282

Oliver, W.T. 1953. Studies in phenothiazine. I. Some in vitro effects of phenothiazine on Heterakis gallinae. Canad. J. comp. Med. 17:435-447.

Olivier, L., Allen, R.W. and Hardcastle, A.B. 1943. Removal of caecal worm, Heterakis gallinae from chickens by feeding phenothiazine in the mash. Vet. Med. 38: 384-386.

Osipov, A.N. 1957. The ability of Heterakis gallinarum eggs to survive in cold conditions. Trudi Moskovskoi Veterinarnoi Akademic. 19: 350-355 (In Russian).

Osipov, A.N. 1957. The biology of Heterakis of chickens. Ibid. 19: 21-29. (In Russian)

Quack, M. 1913. Ueber den feineren Bau der Mitteldarmzellen einiger Nematoden. Zellforsch. 11: 1-50.

Railliet, A. and Lucet, A. 1892. Observations et. experiences sur quelques helminths du genrer Heterakis Dujardin. Bull. Soc. Zool. France 17-19: 117-120.

Rauther, M. 1909. Morphologie und Verwandtschaftsbeziehungen der Nematoden. Ergeb. & Fortschr. Zool., 1: 491-496.

Rauther, M. 1918. Mitteilungen zur Nematodenkunde. Zool. Jahrb. Abt. Anat. 40: 441-514.

Riley, W.A. and James, L.G. 1921. Studies on the chicken nematode, Heterakis papillosa, Bloch. J. Amer. vet. med. Ass. 22: cf. Clapham, P.A. 1933. J. Helm. 11: 67-86.

Roberts, F.H.S. 1937a. Studies on the biology and control of the large roundworm of fowls, Ascaridia galli (Schrank 1788) Freeborn, 1923. Bull. Anim. Hth. Stat. 2: 1-106.

Roberts, F.H.S. 1937b. Studies on the life-history and economic importance of Heterakis gallinae (Gmelin) Freeborn, the caecal worm of fowls. Austr. J. exp. Biol. med. Sci. 15: 429-439.

Roth, K. 1903. Beitrage zur systematic und Biologie der Heterakis inflexa vesicularis und maculosa und zur. Anatomie und physiology vorkmmen Zellen. Diss. 40 pp Leipzig.

Savchenko, M.E. 1959. Seasonal dynamics of Heterakis infections in domestic fowls in Krinonozh. Veterinariya 36: 43 (Abstract).

Schneider, A. 1860. Ueber die Muskeln und Nerven der Nematoden. Arch. Anal. Physiol. u. Wiss. Med. 224-242.

Schneider, A. 1866. Monographic der Nematoden. 357 pp. Berlin.

Schneider, K.C. 1902. Lehrbuch der vergleichenden Histologie der Tiere. 988 pp Jena.

Scott, J.W. 1913. A new means of transmitting the fowl nematode, Heterakis perspicillum. Science 38: 672-673.

Shumard, R.F. and Eveleth, D.F. 1955. A preliminary report on the anthelmintic action of piperazine citrate on Ascaridia galli and Heterakis gallinae in hens. Vet. Med. 50: 203-205.

Siebold, C. Th. von. 1848. Lerbuch der vergleichenden Anatomie der wirebellosen Tiere. Berlin.

Smith, P.E. 1953. Life history and host parasite relationship of Heterakis spumosa, in nematode parasite in the colon of the rat. Amer. J. Hygine 57: 194-221.

Spena, A. 1935. Sulla ascaridiosis dei polli. Clinica vet. Milano, 58: 863-869.

Swales, W.E. 1940. The use of phenothiazine in veterinary parasitology. Canad. J. Comp. Med. 4: 333-334.

Swales, W.E. 1952. Entero-hepatitis (black-head) in turkeys. VIII. Further observations on the use and mode of action of 2-amino-5-nitrothiozole. Canad. J. Res. 16: 57-62.

Tyzzer, E.E. 1934. Studies on Histomoniasis or 'black-head' infection, in the chicken and turkey. Proc. Amer. Acad. Arts and Sci. 69: 189-264.

Uribe, C. 1922. Observations on the development of Heterakis papillosa Bloch. in the chickens. J. Parasit. 8: 167-176.

Van Es, L. and Martin, H.H. 1923. The more important poultry diseases. Bull. agric. exp. Sta. Univ. Nebraska 195. cf. Roberts, F.H.S. 1937. Aust. J. exp. Biol. med. Sci. 15: 36-40.

Vante, R.D. 1963. Aspects of the biology of Heterakis gallinarum (Nematoda) in chickens and host parasite relations. Diss. Abstracts. 24: 1767-1768.

Vogel, R. 1925. Zur Kenntnis der Fortpflanzung, Eireifung, Befruchtung und Furchung von Oxyuris obvelata Bremser. Zool. Jahrb. 42: 243-271.

Ward, J.W. 1947. The life history and bionomics of Heterakis bonasae, a caecal nematode of bob white quail, Colinus virginiansus and Colinus virginiansus texanus. J. Parasit. 33: 24 (Suppl.).

Wehr, E.E. and Olivier, L. 1946. Limitations of phenothiazine in the control of caecal worms and black-head disease of turkeys. Poult. Sci. 25: 199-203.

Wickware, A.B. 1940. Effects of freezing temperatures on the embryonation of eggs and infectivity of larvae of Heterakis gallinae. Canad. J. comp. Med. 4: 110-116.

Wickware, A.B. 1947. The differential blood picture in chickens before and after administration of embryonated eggs of Heterakis gallinae with notes on pathogenicity. Ibid. 11: 78-83.

Yamaguti, S. 1961. Systema Helminthum Vol. 3, parts 1 & 2, New York. (Interscience publishers).

Yorke, W. and Maplestone, P.A. 1926. The nematodes parasites of Vertebrates. London. X + 536 pp.

LIST OF ABBREVIATIONS

amph.	Amphid
am.gl.	Amphidial gland
am.nrv.	Amphidial nerve
an.	Anus
bas.lam.	Basal lamella
bac.lyr.	Bacillary layer
bas. mem.	Basal membrane
cap. c.	Cap cell
cep. pap. nrv.	Cephalic papillary nerve
ceph. pap.	cephalic papillae
cd. al.	Caudal alae
clo.	Cloaca
cu.	Cuticle
cut. lyr.	Cuticular layer
d. nrv. chd.	Dorsal nerve chord
d.gn.	Dorsal ganglion
d.chd.	Dorsal chord
d. gl.	Dorsal gland
dep. ani.	Depressor ani
d. lp.	Dorsal lip
exc. dct. term.	Terminal excretory duct
fbr. lyr. ext.	External fibre layer
germ. c. nu.	Nucleus of the germ cell
int. lu.	Lumen of the intestine

lat. chd.	Lateral chord
lat. gn.	Lateral ganglion
lat. exc. can.	Lateral excretory canal
lat. exc. dct.	Lateral excretory duct
lat. vent. lp.	Latero-venteral lip
lt. rdg.	Lateral ridge
lumb. gn.	Lumbar ganglion
med. fbr. lyr.	Median fibre layer
mat. lyr.	Matrix layer
mus. c.	Muscle cell
marg. mus.	Marginal muscle
marg. nu.	Marginal nucleus
m.	Mouth
nrv. r.	Nerve-ring
nu.	Nucleus
nucl.	Nucleolus
nrv. nu.	Nerve nucleus
oes. lu.	Lumen of the oesophagus
ov. d^1	Anterior oviduct
ov. d^2	Posterior oviduct
oes. int. valv.	Oesophago-intestinal valve
ov^1	Anterior ovary
ov^2	Posterior ovary
ov.	Ova
ph.	Phasmid
part. can.	Partition canal

pa.	papillae
plm.	Protractor muscles of the lips
rct. lu.	Lumen of the rectum
rad. mus.	Radial muscle
rct. gn.	Rectal ganglion
rc. gl.	Rectal gland
rlm.	Retractor muscles of the lips
rls.	Retractor muscles of the long spiculum
rss.	Retractor muscles of the short spiculum
sc.	Spicular canal
sh.	Spicular sheath
ssp.	Short or sinistral spiculum
su.	Sucker
sup.	Sucker papillae
spl. zon.	Sarcoplasmic zone
sph. mus.	Sphincter muscle
sub. bac. lyr.	Sub-bacillary layer
sub. v. gn.	Sub-ventral ganglion
Sub velp.	Sub-ventral lip.
spr.	Sperm
spr. mt.	Spermatozoa
te.	Testis
tl.	Tail
tlp.	Tail papillae
ut^1	Posterior uterus
ut^2	Anterior uterus

valv.	Valve
vulv.	Vulva
v. chd.	Ventral chord
v. gn.	Ventral ganglion
vag. valv.	Vaginal valve
vd.	Vas deferens
vag. ver.	Vagina vera
vag. ut.	Vagina uterina